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The British Mycological Society

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TRANSACTIONS

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Volume 42

Edited by
S. D. GARRETT and GRACE M. WATERHOUSE

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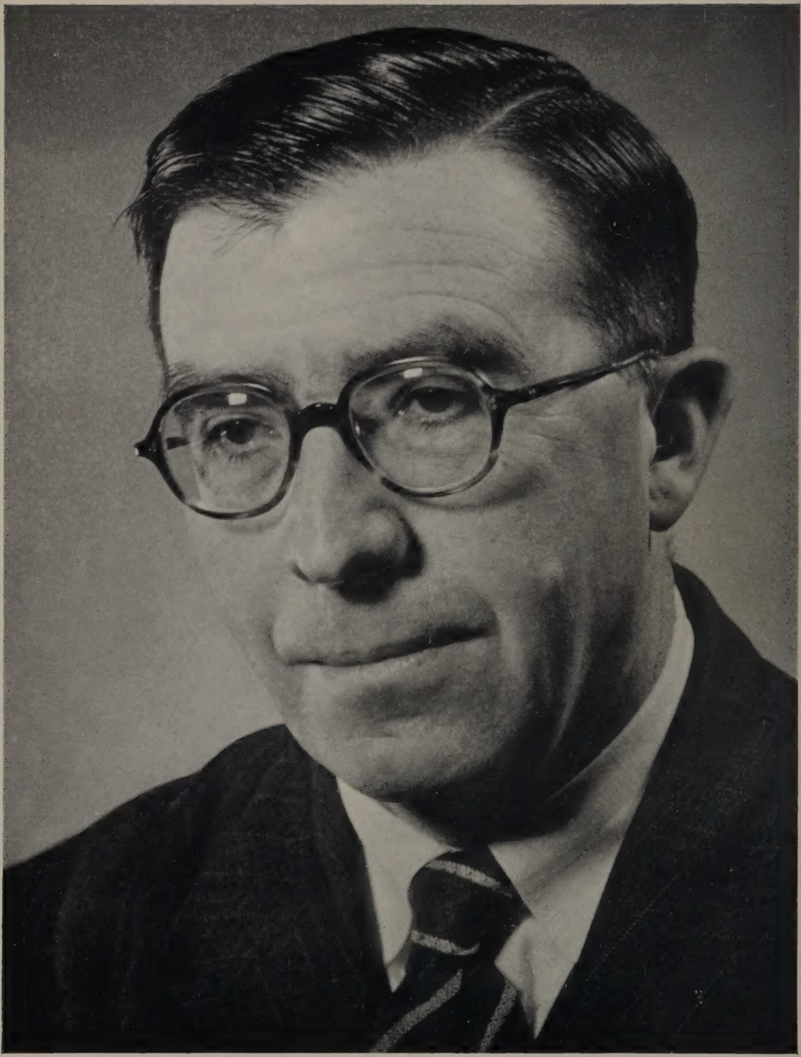

F. B. HORA
President, 1958

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PRESIDENTIAL ADDRESS

QUANTITATIVE EXPERIMENTS ON TOADSTOOL PRODUCTION IN WOODS

By F. B. HORA

(With 7 Text-figures)

> We do not know a thing properly until we know its name.
>
> PROF. GEORGE CROOM ROBERTSON

> The primary object of this book is to enable the reader to determine the names of our indigenous mushrooms, toadstools, etc. At the same time, if a desire to know more than a mere string of names has not been aroused, then I have failed in my object.
>
> GEORGE MASSEE

In Higher Plants, a great deal of ecological work has been and is being done, and each year shows an ever-increasing interest in the essentially experimental approach both in pure and applied botany. In the case of toadstools, the matter is very different: relatively little ecological work has been published (Cooke, 1948, 1953). The work of Wilkins and his associates (1937–46), operating from Oxford, and of Morten Lange (1948), in Denmark, are notable exceptions. Of experimental ecological work on toadstools, I do not think it would be unfair to say that it is conspicuously inconspicuous. In the case of agarics this is, perhaps, understandable. In the first place, it is not easy to discriminate the species involved, and the very nature of agarics places a certain limitation on what can be done by way of sending up packages of them for someone else to name. However, with a suitably designed experiment, I think this difficulty could be overcome. Another reason, perhaps, is that most experimentally minded biologists prefer to carry out their experiments with the maximum control of the environmental conditions and, even when they are not too wedded to their laboratories, may be a little wary of investigating populations which are liable to exhibit a 'patchy' distribution, both in time and in space. What I hope to convince you of by the end of this Address is that a little boldness in the direction of carrying out quite simple experiments, in the virtually uncontrolled conditions of Nature, may yield interesting and unexpected results, suggest work for further experiments, and even produce new ideas.

Had I more time at my disposal, I would like to develop the view that if you want to understand how organisms grow together, you must experiment with them as they grow together. The extrapolation of laboratory results such as from autecological studies, to the field, is often of very limited value and may be misleading. This view has been very well put by Bunting & Cartwright (1957).

Vol. 41, Part 4 was issued 19 December 1958

Having spent some time in acquiring the ability to determine agaric species, my training as a plant physiologist has prompted me to try and find out more about them—and by the experimental approach. Three years ago I laid down some simple experiments in three pine plantations. During the last three toadstool seasons, I have accumulated a vast amount of numerical data. What I propose to do within the next hour is to tell you quite simply what I have been doing and to bring before your notice a few results which, at least, have interested me. The more detailed and elaborate working up of the data must be left for subsequent publications.

Some ten miles towards the south-west of Reading is an area which may be conveniently referred to by the village name of Mortimer. The particular part in which I am interested is a low plateau about 320 feet above sea-level. (The Thames at Reading is about 120 feet above sea-level.) Mortimer is capped by Plateau Gravels which overlies the Bagshot Sands and these in turn rest on the London Clay. One or two shallow valleys have been cut out by small streams. For most of their length, the streams have cut back into the Bagshot Sands which thus form the greater part of the sides of the valleys, but, at lower levels, small areas of London Clay are exposed. The prevailing forestry policy aims at maintaining conifers, mostly Scots pine, on the Gravels, with pedunculate oak on the Bagshot Sands—hence the oaks are confined almost entirely to the valley sides—whilst alders, commonly pollarded, occupy the sides of the streams. My work has been carried out in three Scots pine plantations of about 25, 30 and 80 years' standing. Being derived from the Plateau Gravels, the soil is extremely poor and is clearly podsolized. The acidity ranges from pH 3·3–4·5 (quinhydrone method), but the majority of the values come out around pH 3·5–3·8. In all these plantations the ground flora was sparse: they were, in fact, chosen for that reason, and a carpet of needles was virtually all that one encountered. Peat does, of course, accumulate and is of the *mor* type, with all that that implies (cf. Pearsall, 1952). In each wood, five experimental plots were laid out, each 10 by 20 yards, the corners being marked by oak pegs. In each plantation, the plots were labelled I–V and dressed with the following fertilizers at the stated rates:

I	6 oz. hydrated lime per square yard.
II	3 oz. superphosphate per square yard.
III	3 oz. sulphate of ammonia per square yard.
IV	3 oz. 'Growmore' complete fertilizer per square yard.
V	1½ oz. potassium nitrate per square yard.

The first dressings were carried out in the winter months of 1956. As a control plot in each plantation, I at first decided to have one area of 200 square yards, divided into four parts, each quarter being near one experimental plot. To this end, for each quarter plot, I drove an oak peg into the ground, the idea being to use this as the centre of a circle of area 50 square yards, using a 4-yard length of string as radius. However, numerous counts in the first season soon showed that these small areas were likely to be too much affected by the 'patchiness' I have already referred to, and I abandoned them. Instead, I pegged out, adjacent to each experimental plot, an exactly similar plot, so that each experimental plot had its own control. This, of course, greatly increased the work, resulting in a total of 30 plots to be examined at each visit.

I began collecting for the first time in August 1956. Every week, each plot, both experimental and control, was visited, all agarics (including *Boletus*) were collected, counted and then discarded outside the plots. The counting and collecting presented one or two points on which a decision had to be made. In the first place, one is confronted with the question: what is an individual toadstool? Thus, does one count a tufted species such as *Hypholoma fasciculare* as a single unit? In all such cases (and they were very few), I have taken each stem as representing a single individual. Another point concerns collecting. As already implied, I removed the specimens from the plots each week. Morten Lange (1948), however, counted what he judged to be fresh individuals at each visit and did not remove the fruitbodies 'partly in order to avoid uncontrolled interference in the growth of the mycelium and thus perhaps artificially altering the fruiting later in the season or in the following year'. He admits that this method has its own complications especially in the case of long-lived fruit-bodies which may get counted two or three times. However, the purpose of Lange's work was very different from mine, nor was he using experimental and control plots in the way I am. Whilst I admit I do not know what effect removal of a fruit-body from its mycelium may have on the production of further fruit-bodies, I have assumed that any effect will apply equally to control and experiment.

Table 1. *First-year list of species in 25-year-old pine wood*

Amanita rubescens
A. spissa
Boletus badius
B. chrysenteron
B. luteus
B. subtomentosus
B. variegatus
Clitocybe langei Sing.
(=*C. vibecina* ss. Lange)
Collybia cirrata
C. maculata
Cortinarius albo-violaceus
C. cinnamomeo-lutescens Henry
(=*C. cinnamomeus* Auct.)
C. hemitrichus
C. malachius
C. semi-sanguineus
C. suillus
Flammula spumosa
Galera hypnorum
Gomphidius roseus
G. rutilus
Hydnum auriscalpium
Hygrophorus hypothejus
Inocybe acuta
I. boltoni
I. umbrina
Laccaria laccata
Lactarius hepaticus
L. rufus
Marasmius androsaceus
M. myosurus
Mycena epipterygia
M. galericulata
M. galopus & var. *nigra*
M. sanguinolenta
Nolanea cetrata
Omphalia maura & f. *alba* Kühner
Paxillus atrotomentosus
P. involutus
Russula emetica
Tricholoma rutilans
T. scalpturatum

Binomials without author citation are those of Pearson & Dennis (1948).

Most of the results here given are derived from the 25-year-old pine plantation. Table 1 gives a complete list of agaric species which were gathered from the 10 plots in this plantation in the year 1956. So far as I was able to find out, no additional species occurred *outside* these plots.

I have said that I collected and counted all agarics present. This was not quite true. *Marasmius androsaceus* defeated me. It was not so much its small size—it is about as small as *Mycena galopus* and *M. sanguinolenta*, and these I gathered without much trouble—but, in spite of its horse-hair-like black stem, it seems very well camouflaged and I found myself constantly missing it. I should also mention that I was unable to resist collecting and recording *Hydnum auriscalpium*. It is scarcely an agaric, but it has always appealed to me as a particularly attractive toadstool.

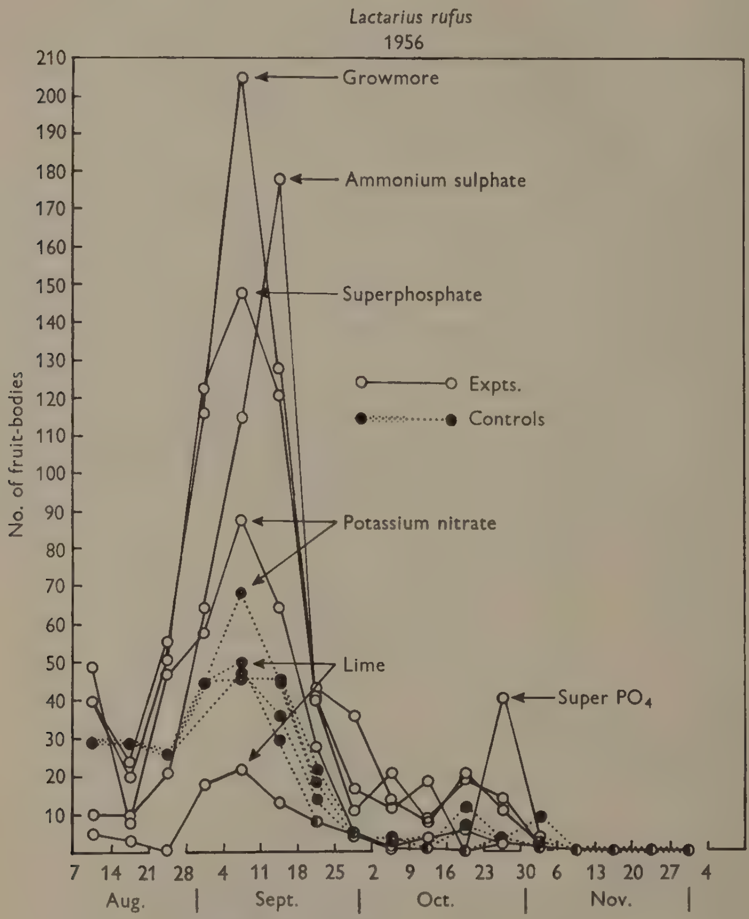

Fig. 1. *Lactarius rufus*. Weekly counts of fruit-bodies.

From this list, results are given for three species, individuals of which appeared in large numbers: *Lactarius rufus*, *Paxillus involutus* and *Omphalia maura*. The first two species are common and well known, the last much less so, unless one knows what to do! Fig. 1 gives the weekly totals for *L. rufus*. I have not indicated the control plots which were adjacent to their respective experimental plots, except in the case of potassium nitrate and lime. The initial drop which is apparent in the experimental plot at the second week's collection is, of course, due to the fact that the *first* week's collection represents more than 1 week's growth of fruit-bodies. I had also to consider this point in connexion with the first 4 weeks' control data. It will be recalled that, to begin with, I set up only one control in each plantation, but divided it into four equal circular areas of 50 square yards. The first four control points on the graph (joined by three rows of dotted lines) represent the sum of the gatherings of the four quarters for each of the first 4 weeks. When I set up a control adjacent to each experimental plot, I was faced with the same situation on the fifth week of each control plot as occurred in the first week of each experimental plot; that is, there was more than 1 week's growth of fruit-bodies. I accordingly made some rough estimates of the lifetime of fruit-bodies of *L. rufus*. I considered it to be between 3 and 4 weeks. Morten Lange (1948) gives 4 weeks. However, so as not to bias the control results too much in my favour, I divided the figures for the fifth week's controls by three, and the results are the ones plotted. It is quite clear from this graph that Growmore, ammonium sulphate and superphosphate, at least, are remarkably effective in stimulating the fruit-body formation of *L. rufus*, whilst lime has a depressing effect. The last result is, of course, not surprising, since *L. rufus* could certainly be classed as a 'calcifuge' species. The effects of potassium nitrate are intriguing and suggest possibilities for further experiment, more especially when its effects on *P. involutus* are also taken into account. Any stimulating effect is small compared with the other three fertilizers. I think it quite likely that nitrate nitrogen is not a suitable source of nitrogen for toadstools on these soils. It is well known that *mor*

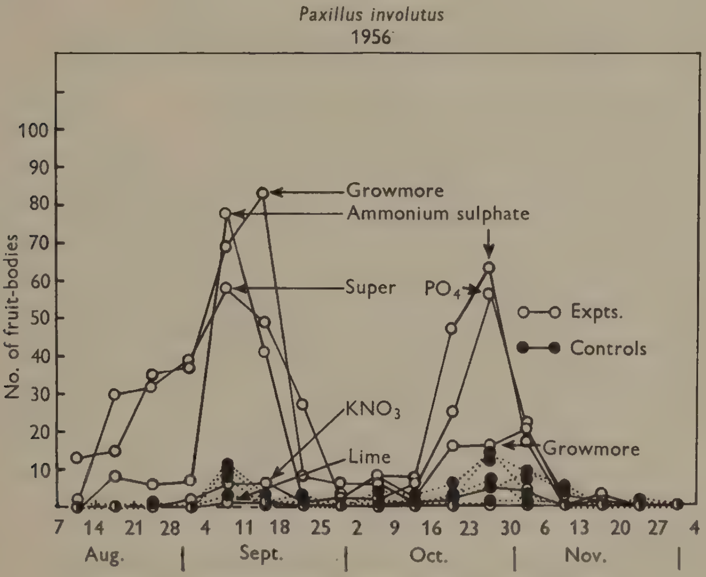

Fig. 2. *Paxillus involutus*. Weekly counts of fruit-bodies.

soils do not normally contain nitrates, and I strongly suspect that most of the organisms of such soils have their nitrogen metabolism geared to an external supply of nitrogen in the ammonium form. In this context, it is perhaps significant that both Growmore and ammonium sulphate are very much more effective in stimulating fruit-body production than is potassium nitrate. Growmore itself has its nitrogen in the ammonium form. I should perhaps point out that the relative amounts of nitrogen supplied are not a factor. The percentage amounts of nitrogen in Growmore, ammonium sulphate, and potassium nitrate are respectively 7, 21 and 14. Since the dressing with potassium nitrate was at half the rate of the others, the amount of nitrogen actually added was identical with that added as Growmore.

Many fungi have been classified in terms of whether they can use nitrate or ammonium nitrogen (Lilly & Barnett, 1951; Hawker, 1950). Much of this work has been carried out with micro-fungi, but some results are available for agarics. I note that my *bête-noire*, *Marasmius androsaceus*, is able to utilize only ammonium or organic nitrogen, but not nitrates—in fact, as I would expect. Of course, even the nitrate users must be able to reduce the nitrate to the ammonium level, for, in Prianischnikov's well-known aphorism: 'Ammonia is the alpha and omega of nitrogen metabolism.' As a question in pure plant physiology, I should be interested to know if those organisms which are unable to use nitrate are unable (*a*) because they lack the enzyme nitrate reductase, which brings about the chemical reduction of nitrate, or (*b*) because of the lack of molybdenum which is an essential trace element for the activity of that enzyme. Available amounts of this element are notoriously deficient in acid soils. If the enzyme is present and active, the possibility of *nitrite* toxicity should be considered.

The graph also shows a small secondary maximum later in the season on the superphosphate plot. I at first thought that this might be a simple collecting hazard. That is to say, on the previous week, it so happened there were no fruit-bodies, and thus the small secondary maximum resulted from 2 weeks' growth of fruit-bodies. Halving the number would have brought the two points at about the same level as in previous weeks. However, when taken in conjunction with the results of *P. involutus* (Fig. 2), I incline to it being a genuine 'spurt'. The week in which it occurs (23–30 October) may not be coincidence.

The corresponding results for *P. involutus* are, for the most part, very similar, except for the striking bi-modal distribution. Growmore, ammonium sulphate and superphosphate in the first maximum are strongly stimulating, and in that order, which is the same order as for *L. rufus*. Potassium nitrate is, if anything, inhibitory and so is lime. Both these inhibitory effects were in evidence in the second and third seasons. It is very tempting to see in the two very distinct maxima something akin to the zonation effect so often seen in micro-fungal cultures (cf. Hawker, 1950). I cannot now enlarge on this observation, and must content myself with pointing out that the secondary maximum occurs between 23 and 30 October—exactly the same time as the small 'spurt' shown in the superphosphate plot of *L. rufus*.

I now turn to the third species: *Omphalia maura*. As regards the first year's results, I have only this to say: no more than thirteen fruit-bodies occurred throughout the whole 30 plots, and all of them were on the lime plot of the 25-year-old plantation. I shall return later to the subsequent history of this species.

Now, at the end of the first season's experiments, I had to make a decision that was not easy. Should I continue with the diversity of fertilizer treatments, or should I sacrifice such diversity in favour of some sort of replication? In the end, the following procedure was adopted. In the early part of 1957, I redressed the lime plots as before and at the same rate, but I spread Growmore over all the remaining experimental plots at the rate of 3 oz. per square yard. In the following toadstool season I harvested the crop as before. The same fertilizer treatment was given in the early part of this year (1958) and again harvesting was along the same lines.

Figs. 3 and 4 give the grand totals for the three seasons for the two species *L. rufus* and *P. involutus*. (The heights of the histograms, representing the number of fruit-bodies, are all measured from the zero base-line.) The differential effect of the various treatments is well brought out. It is clear that lime is killing off the fruit-body production of *L. rufus* and also, but perhaps less obviously so, that of *P. involutus*. At this point, it will be convenient to distinguish the plot which was first dressed with potassium nitrate as the 'Nitrate–Growmore Plot', the remaining three plots as simply the 'Growmore Plots'. In the case of *L. rufus*, the 'Growmore Plots' continued to cause stimulation, remarkably so in 1956, but this was less marked in the 'Nitrate–Growmore Plot'. This strengthens, I think, the suggestion already made that nitrate nitrogen is not such a good source of nitrogen as is ammonium nitrogen on acid soils. Much the same results are seen for *P. involutus*. In the 'Nitrate–Growmore Plots', there was definite inhibition, except in the year 1957. But this year was, for *P. involutus*, a real bumper year, at least in conjunction with the appropriate fertilizer treatment. It is a common experience amongst agaricologists that certain years are more favourable to one species, in terms of number of fruit-bodies produced, than to another. 1957 was a particularly good year for *P. involutus*, but a poor year for *L. rufus*—both in terms of the 'Growmore Plots'. On the other hand, 1956 was a good year for *L. rufus* but a poor one for *P. involutus*—again in terms of the 'Growmore Plots'. Presumably, this is an example of inter-specific adaptation and competition for the available food supply. If conditions are favourable for the increase of one species, then it increases at the expense of others which overlap in time. *Big* increases in two vigorous species such as the histograms show are not, therefore, to be expected in the same year. It is interesting to note that in this year (1958), *L. rufus* and *P. involutus* were only moderately stimulated in the experimental plots other than lime, and *P. involutus* not at all in the 'Nitrate–Growmore Plot'. Although I have not the time at my disposal to give the evidence, I can say that these big increases in either *P. involutus* or *L. rufus* have been accompanied by a progressive *decrease* not only in the number of other species, but also in the numbers of individuals of other species. All this seems to me to show how toadstool populations resemble human populations in terms of the principle enunciated by the Rev. Thomas Malthus: they increase up to the limit set by the available means of subsistence.

One final word on these histograms. Comparing the relative areas of blacked-out histograms in the two species, that is, the two sets of control plots, it is plain that, under the normal conditions obtaining in the plantation, *L. rufus* is a more successful toadstool than *P. involutus*.

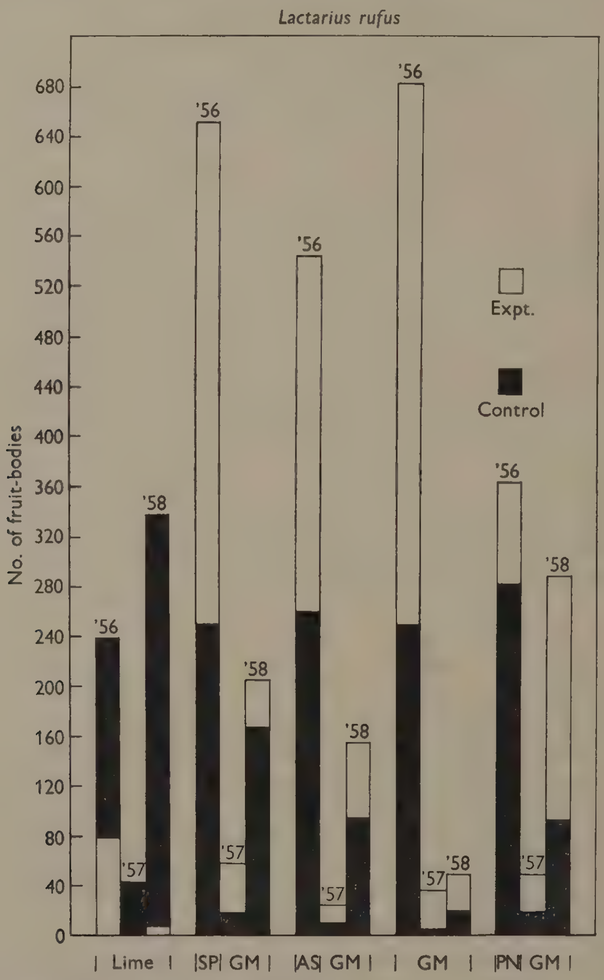

Fig. 3. Grand totals of *Lactarius rufus* on all the plots of the 25-year-old plantation. Heights of histograms in both control and experimental plots are measured from the zero base-line. 1956, 1957, 1958 are the years of the three toadstool seasons. SP = superphosphate; AS = ammonium sulphate; PN = potassium nitrate; GM = Growmore.

I must now pass on to the final species, *Omphalia maura*. I have already given a preliminary note in a letter to *Nature* (Hora, 1958). It will be recalled that I said that only thirteen individuals occurred on the experimental lime plot of the 25-year-old plantation. It was absent on all the other plots in the other two woods and I have not seen a specimen in the Mortimer area for many years. Fig. 5 shows what happened in 1957. It occurred in all three plantations but only on the experimental lime plots with the maximum in the 80-year-old plantation. The maximum weekly taking was some 2500 individuals. The respective grand totals are

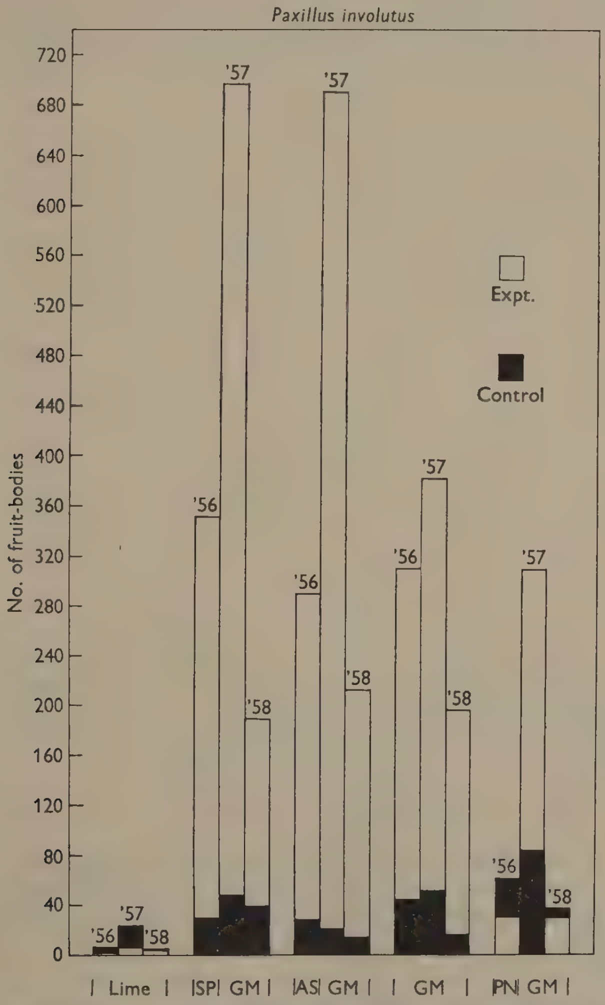

Fig. 4. Grand totals of *Paxillus involutus* on all the plots of the 25-year-old plantation. Symbols and abbreviations as in Fig 3.

also impressive, especially when viewed against those of the controls. However, in case it might be thought that this performance is an all-time record, Fig. 6 shows what it really can do. The grandest total is here over 25,000 individuals, but this time on the 25-year-old plantation. The maximum weekly taking was nearly 7800 individuals. The 39 specimens on the control were all on the margin between the experimental and control plots and thus without doubt owe their existence to some lime which came down on the wrong side of the dividing line. They, therefore, properly belong to the experimental plot.

Omphalia maura is a species which is virtually confined to burnt ground (Moser, 1949). I do not mean the sort of burnt ground that results from the firing of a plantation (which happens too often at Mortimer), but ground which has recently been the site of something approaching a bonfire. This is also true of its continental distribution and I have received letters to that effect from France, Switzerland, Holland and Austria. I do not know what the American experience is. The question at once arises: what is common between lime and burnt ground? Wood ashes will, of course, raise the mineral status of the soil and when fresh the presence of soluble potassium carbonate will result in values of pH 10 or even more. Subsequent leaching out of the potassium will still leave the relatively insoluble carbonates of calcium and magnesium which result in a pH = 8·5 or thereabouts, the same, in fact, as a typical limestone soil. One might expect then to find *O. maura* on a limestone soil. But this is not the general experience. It occurs on acid soils, mostly in coniferous woods where there has recently been considerable contamination with wood ashes. I have never found it on limestone soils, not even where there has been burning. A hypothesis consistent with these facts would be that the production of fruit-bodies depends on the sudden addition of alkali to an acid soil.

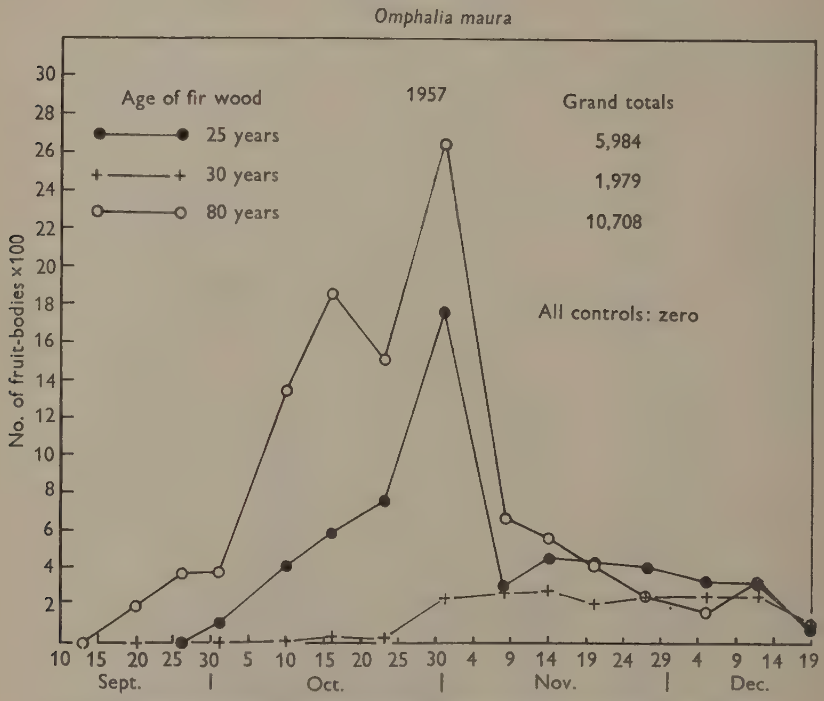

Fig. 5. Weekly counts of *Omphalia maura* on all three plantations, 1957 season.

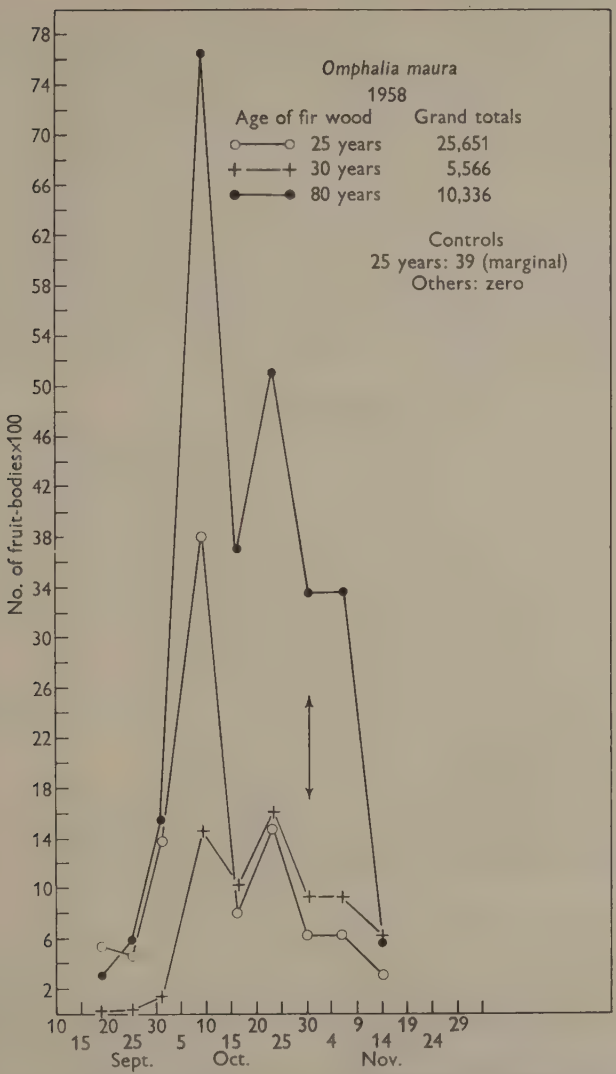

Fig. 6. Weekly counts of *Omphalia maura* on all three plantations, 1958 season. The vertical arrow indicates that no count was made that week and the points plotted were obtained by dividing the subsequent week's count by 2.

Whether lime or any other alkali will do, I am at present investigating. I have no information whether or not the fruit-bodies arise from perennial mycelium or from spores brought by wind. I rather suspect the latter, because it is very noticeable that the fruit bodies seem to arise most frequently directly from lime which has matted together a few fir needles, as if the spores had just settled on and germinated. I must admit, however, that the appearance of a few white forms of *Omphalia* in the same places in two successive seasons rather points to a perennating mycelium. If the sudden addition of alkali is what is wanted, then, I take it, fruit-body production on the lime plots will steadily decline when successive seasonal additions of lime eventually begin to saturate the substratum, preventing any further sudden increases in pH. It is possible that the fruit-bodies arise from the needles which fall each year, In this case, fruit-bodies may continue to arise, so long as they are dusted with lime each year.

The conditions affecting fruit-body formation in *O. maura* would appear to be different from such species as *Coprinus lagopides* Karst. and *C. gonophyllus* Quél., whose fruit-bodies occur on burnt ground as well as on plaster ceilings (Orton, 1957), where in the latter habitat, presumably, no sudden addition of alkali is involved. It is worth noting that *C. lagopides* has turned up in the 30-year-old plantation on the experimental lime plot. I also know it from chalk soil just north of the Thames at Reading.

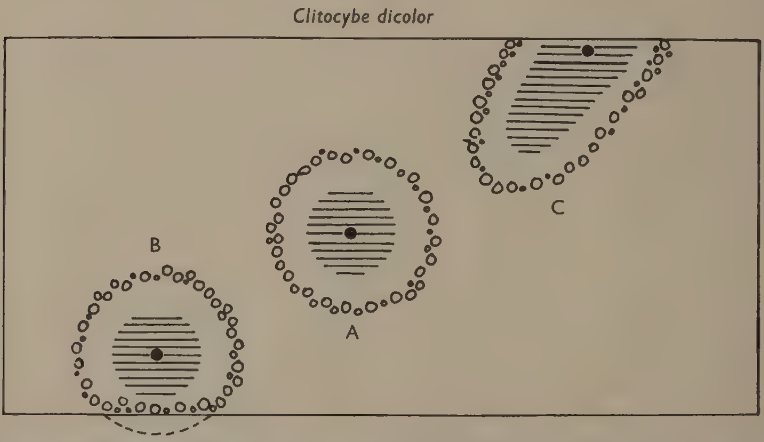

Fig. 7. Fairy rings of *Clitocybe dicolor* on the lime plot of the 80-year-old plantation. The diameter of the rings is about 2 feet. The ellipse is drawn roughly to the same scale. 1958 season.

This interesting connexion between burnt ground and lime is not confined to the agarics. Two very pretty, more or less blue, discomycetes have also appeared on the lime plots of the 25- and 80-year-old plantations. I sent them to Mr Graddon for identification, at the same time asking where he would expect to find them. He at once recognized them as *Galactinia praetervisa* (Bres.) Boud. and *Aleuria lilacina* Boud. Their usual habitat? Burnt ground!

I believe that *Omphalia maura* is a species well worth investigating in the laboratory and I hope to get it into culture. In addition to its obvious nutritional interests, it also shows interesting pigment variations. There seem to be at least three colour forms. In the water-soaked condition, the vast majority have a black cap. There is also a completely white form which has always come up in the same places, and a more or less sepia-coloured form. One final point: the species survives very well throughout winter, and last year specimens continued to come up until almost Easter.

Fig. 7 is based on an observation made in November 1958. *Clitocybe dicolor* first appeared on the limed plot in the 80-year-old plantation in

1957, when a few specimens were collected. This year it occurred on the same plot in the form of two rings and an incompletely closed ellipse. I think it possible that the latter may have arisen from two centres of inoculation. The central ring is the sort of thing that is commonly encountered. Ring B was complete, but at the margin of the lime plot the fruit-bodies followed the plot margin in a straight line, forming a chord to the circle. Presumably, one necessary factor for the formation of fruit-bodies is the presence of lime. In C, however, the fruit-bodies were unable to close the ellipse. All this seems to be accounted for in terms of the distance of the original point of inoculation from the plot margin. We can then imagine something like this: young mycelium + appropriate environmental conditions = fruit-bodies. In A, the left-hand side of the equation is fulfilled and fruit-bodies appear, In B, the original point of inoculation has not been too near the margin to prevent young mycelium being present at the margin. The dead, dying or stale mycelium has not yet reached the margin. Conditions, therefore, are all right for fruit-body formation, but not outside the plot, where one of the appropriate conditions, namely, lime is absent. In C, the point of inoculation was too near the margin. The dead, dying or stale mycelium was already at the margin. Thus the appropriate environmental conditions were present, but young mycelium was lacking. I hope this simple observation may add something to the account of fairy rings according to Buller (1922) and Ramsbottom (1953). If I could follow the impressive mathematics of Parker-Rhodes (1955), I might find that it added something to their kinetics.

I began this Address with two quotations. I do not think I can do better than end it with a third:

SINE EXPERIMENTIA NIHIL SUFFICIENTIER SCIRE POTEST

This motto occurs over the door of the entrance to the Daubeny laboratory at Oxford which was formerly part of the University Department of Botany.

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Note added in proof

By the end of January 1959, numbers of *Omphalia maura* had increased by at least the following amounts:

25-year-old pinewood, 7,000.
30-year-old pinewood, 1,000.
80-year-old pinewood, 3,000.

Trans. Brit. mycol. Soc. **42** (1), 15–26 (1959).

THE CULTURE OF *PHYTOPHTHORA INFESTANS* IN ARTIFICIAL MEDIA

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A prerequisite for possible biochemical genetical studies on *Phytophthora infestans* is a simple 'minimal medium' of known composition. A liquid medium containing only mineral salts, D-glucose, asparagine and thiamine seems likely to be suitable, but the growth of the isolate of Race 2, 4 with which most of this work was done showed considerable variation on this medium. The amount of growth was shown to be affected by the nitrogen concentration in the medium and by the size of the initial inoculum used, there being an interaction between these two factors. A compound formed during prolonged autoclaving of a mixture of glucose and asparagine was able to decrease or suppress growth on the 'minimal medium' even in the presence of yeast extract. The presence of this compound probably accounts for the decrease of growth with an increase in the time of autoclaving of the medium, and it is probably formed in small amounts during normal autoclaving. Separate autoclaving of the N source caused a very slight increase in growth.

INTRODUCTION

An essential prerequisite for an investigation of the mechanism of variation in *Phytophthora infestans* is a defined medium of the simplest possible constitution on which the fungus will grow and sporulate satisfactorily, and on which single zoospore isolates will develop in a reasonable time.

P. infestans was grown in synthetic culture media by Jones, Giddings & Lutman (1912), who concluded that organic N, in this case asparagine, as well as $MgSO_4$, K_2HPO_4 and possibly KCl were essential for growth. Payette & Perrault (1944) grew *P. infestans* in a liquid mineral-dextrose medium containing asparagine, and found that thiamine (opt. 0·2 μg./ml.) was essential.

The growth of *P. infestans* in synthetic media has also been investigated by French (1953), who found that two physiologic races differed in their ability to utilize various N sources, notably asparagine and a mixture of amino acids (aspartic acid, glutamic acid, arginine, glycine and alanine). The two races also differed in their response to growth factors present in raw sugar and yeast extract. More recently, Sakai (1955*a*, *b*, 1956) has contributed to our knowledge of the nutrition of *P. infestans* in synthetic culture solutions.

In the work reported here the medium defined by French was used as a basis for further investigation, but it early became apparent that the growth of *P. infestans* varied greatly between experiments in which the same or similar nutrients were provided. The investigation of the reasons for this variation is reported in the following pages.

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* Formerly Angela M. Clayson.

MATERIALS AND METHODS

Isolates of Phytophthora infestans *used in nutritional experiments*

In most of the experiments described below, the isolate of *P. infestans* was one originally obtained from Miss M. Keay (then at the Potato Genetics Station, Cambridge) as Race 2,4. It subsequently lost pathogenicity in culture, but continued to spore fairly well and was used throughout for the sake of consistency. Its behaviour was subsequently compared with that of isolates of Race 4 (isolated from infected Majestic plants) and Race 1,4 (obtained from Miss M. Kostrowicka, of the National Institute of Agricultural Botany, Cambridge).

Maintenance of stock cultures

Pure cultures of *P. infestans* were kept on slopes of French bean agar (150 g. frozen French beans and 15 g. agar per l. distilled water, autoclaved at 10 lb. for 10 min.) and subcultured at fortnightly intervals to maintain the fungus in a vigorous condition. Spore suspensions for inoculation purposes were obtained from cultures grown on agar slopes in 6 oz. medicine bottles. The sporangial suspension was either used as inoculum immediately, or kept at a temperature of 10–15° C. for about 2 hr. to allow liberation of zoospores. The latter procedure was usually only successful if the sporangia came from a culture which had previously been kept at 10–15° C. for several days.

Single spore isolates

Single spore isolates of the fungus were used unless otherwise stated, and were prepared as follows. A suspension containing zoospores was diluted to give about twenty zoospores per drop. Drops of the suspension were then placed at one side of a Petri dish containing a thin layer of agar, and the dish was tilted so that streaks of spore suspension ran across it, thus separating most of the zoospores. This was found to cause less damage to the zoospores than streaking the suspension across the surface of the agar with a wire loop. The medium usually used was the basal medium described below with the addition of 0·5 % (w/v) Bacto yeast extract and 2·5 % (w/v) agar. Basal medium was also used with the following additions (%, w/v): asparagine, 0·1; peptone, 0·1; yeast extract, 0·1; agar, 2·5 and vitamin mixture. The composition of the vitamin mixture was: riboflavin, 10 mg.; nicotinamide, 10 mg.; *p*-aminobenzoic acid, 1 mg.; pyridoxin-HCl, 5 mg.; aneurin-HCl, 5 mg.; biotin, 0·02 mg.; Ca-pantothenate, 20 mg.; choline chloride, 20 mg.; inositol, 40 mg.; folic acid, 1 mg.; distilled water, 10 ml. One ml./l. of this mixture was used (see Pontecorvo, 1953).

The cultures were then incubated at 22·5° C. for 3–4 days to allow good germination of the zoospores. Single germinated spores, together with a small piece of agar, were then cut out under a binocular dissecting microscope and transferred to tubes containing 2 ml. of one of the media described above, but without agar. Before being transferred to a tube, each spore was examined under the low power of the compound microscope to check that only one was present. After about 2 weeks growth in the liquid medium at 22·5° C., a piece of mycelium could be transferred to a slope of bean agar and grown on in the usual way.

General procedure used in nutritional experiments

Liquid culture experiments were conducted as follows. Conical flasks (150 ml.) containing 25 ml. culture solution were used in all experiments. All glassware was acid washed before use. The basal medium had the following composition (%, w/v): KH_2PO_4, 0·05; $MgSO_4.7H_2O$, 0·025; D-glucose, 2·5; thiamine hydrochloride, 0·0001; distilled water.

The concentrations of the N sources were calculated to give the required total N, which varied in different experiments. The pH of all media was determined with a Marconi pH meter, and adjusted to 5·5 before autoclaving. The media were autoclaved at 10 lb. for 10 min. unless otherwise stated. The concentration of spores in the suspension to be used as inoculum was determined using a Fuchs-Rosenthal haemocytometer. Each flask was then inoculated with 0·25 ml. spore suspension. The cultures were incubated at 22·5° C. for 2 weeks, after which the mycelium from each flask was filtered off on a previously dried and weighed 4·25 cm. Whatman no. 54 filter-paper, dried overnight at 80° C. and weighed. The mycelial dry weights given are means of four replicates, correct to the nearest mg.

EXPERIMENTAL RESULTS

In early experiments a comparison was made of the amino acids, aspartic acid, glutamic acid, arginine, alanine and glycine, singly and as mixtures, with asparagine and glutamine, as sole sources of N for *P. infestans*. The total N concentration in the medium was 0·05 % in all these experiments. Growth occurred to a greater or less extent on all the amino acids and amides mentioned, with the exception of glycine, but the relative amount of growth on the different N sources varied from one experiment to another. However, asparagine and glutamine were generally the best single sources of N, although growth was occasionally very poor even on these.

In these preliminary experiments, inoculum had been used which did not come from a single spore isolate, and it was thought that genetic variation within the isolate might be causing the irregular results. Five single spore isolates derived from the isolate of Race 2, 4 mentioned above were then grown with different N sources (asparagine, glutamine, aspartic acid and alanine) and with different concentrations of asparagine, but no differences in the behaviour of these different isolates could be detected. It was, however, noted that two of the isolates spored sparsely and inoculum prepared from these had a low spore density. Such inoculum invariably appeared to give a limited development of mycelial growth in the culture fluid.

It was therefore decided to investigate further the causes of variation in the amount of growth of one single spore isolate of *P. infestans*, as shown by mycelial dry weight, when grown on a single medium, in this case the basal medium with asparagine as a source of N. Growth of this isolate on asparagine was subsequently compared with that on other amino acids, and with the growth of isolates of two other races.

Effect of initial pH *of medium*

As a check on the practice of adjusting the pH of all media to 5·5, an experiment was set up to investigate the effect on growth of the initial pH of the medium. Asparagine was added to the basal medium to give 0·02 % N. The medium was unbuffered and the pH was adjusted with hydrochloric acid and sodium hydroxide to give values from pH 4·0 to 7·0. The medium was autoclaved at 10 lb. for 10 min. The inoculum was equivalent to *c.* 13,000 sporangia/25 ml. (Table 1).

Table 1. *Effect on mycelial dry weight of* Phytophthora infestans *of initial* pH *of culture solution*

pH of culture solution			
Initially	After autoclaving	After 16 days	Mycelial dry weight (mg.)
4·0	4·0	4·2	4
4·5	4·6	6·7	12
5·0	5·0	7·2	26
5·5	5·2	7·2	24
6·0	5·8	7·2	20
6·5	6·3	6·7	6
7·0	6·7	6·7	2

It was concluded that, on this medium, *P. infestans* was able to begin growth only over a very small range of pH values, between approximately 5·0 and 6·0. In subsequent experiments, therefore, the pH of the culture solutions was adjusted to 5·5. The pH often dropped slightly during autoclaving but rarely fell below 5·0.

Effect of certain growth factors

Although the only growth factor for which *P. infestans* is known to have an absolute requirement is thiamine, it was decided to investigate the effect on growth of this fungus of various supplementary growth factors. These were the vitamin mixture as described above, used at 1 ml./l. (Pontecorvo, 1953), yeast nucleic acid hydrolysate, used at 3 ml./l. of medium, and Bacto yeast extract at 0·5–0·0005 %. Thiamine was included in the basal medium and all media were autoclaved at 10 lb. for 10 min. The inoculum was equivalent to *c.* 20,000 sporangia/25 ml. (Table 2).

The control value here is somewhat lower than has been obtained in other experiments using a comparable inoculum size. All the growth factors increased growth substantially, although they were not essential.

Effect of the carbon source

The 2·5 % (w/v) D-glucose in the basal medium was replaced by a series of other sugars, pectin and potato starch as shown in Table 3, and asparagine was added to give 0·02 % N. The media were sterilized by steaming

Table 2. *Effect on mycelial dry weight of* Phytophthora infestans *of growth factors added to basal medium with asparagine*

Growth factor added	Mycelial dry weight (mg.)
None	11
Yeast extract 0·5 %	57
Yeast extract 0·05 %	68
Yeast extract 0·005 %	41
Yeast extract 0·0005 %	21
YNA hydrolysate	45
Vitamin mixture	36
YNA hydrolysate and vitamin mixture	43

Table 3. *Effect on mycelial dry weight of* Phytophthora infestans *of the source of carbon in the medium*

Carbon source in addition to asparagine	%, w/v	Mycelial dry weight (mg.)
None	—	8
D-Glucose	1·0	54
D-Glucose	2·5	52
D-Glucose	5·0	28
Sucrose	2·5	28
D-Fructose	2·5	2
L-Arabinose	1·0	6
D-Xylose	1·0	0
Raffinose, $5H_2O$	1·0	6
Pectin	0·5	7
Potato starch	2·5	—

for 1 hr. on three successive days. The inoculum was equivalent to *c.* 39,000 sporangia and zoospores/25 ml. (Table 3).

The greatest amount of growth was given by 2·5 and 1 % glucose and so 2·5 % was continued as the standard concentration for further experiments. None of the other sugars used, with the exception of sucrose, was able to support any more growth than that obtained when the sole carbon source was the asparagine. It was not possible to detect any growth on potato starch because the medium was too opaque.

Effect of variations in nitrogen concentration and inoculum size

The comparison mentioned above of the amount of growth made by different single spore isolates gave some indication of the importance of inoculum size in determining the dry weight of mycelium formed in a given time. A factorial experiment was therefore devised in which six replicate flasks in each of four series, containing basal medium plus asparagine giving 0·005, 0·01, 0·02 and 0·05 % N, were inoculated with spore suspensions of decreasing concentration. Estimates of the number of spores present at the higher dilutions were made by calculation and not by direct count. Culture media were autoclaved at 10 lb. for 10 min. (Table 4).

It may be seen that with a larger inoculum the optimum N concentration seems to be in the region of 0·01–0·02 %, but appreciable growth

Table 4. *Effect of nitrogen concentration and inoculum size on mycelial dry weight of* Phytophthora infestans

Approx. no. sporangia per 25 ml. culture solution	Mycelial dry weight (mg.)			
	0·005 % N	0·01 % N	0·02 % N	0·05 % N
56,000	35	45	49	34
28,000	33	41	49	26
11,200	30	42	37	10
5,600	35	36	32	9
1,000	28	21	16	0
200–500	26	32	16	1

does occur at 0·05 % N. At low inoculum levels, less than about 10,000 spores/25 ml., growth seems to be reduced or even suppressed at 0·05 % N, although it is still fairly good at 0·005 and 0·01 % N. In other similar experiments the general pattern of results was the same, but in an experiment with a mixture of sporangia and zoospores as inoculum, the inoculum level at which growth was reduced at 0·05 % N was rather higher (*c.* 20,000 spores/25 ml. culture solution).

A further experiment was carried out, but using glutamine as the source of N (Table 5).

Table 5. *Effect of nitrogen concentration and inoculum size on mycelial dry weight of* Phytophthora infestans

Approx. no. sporangia and zoospores per 25 ml. culture solution	Mycelial dry weight (mg.)			
	0·005 % N	0·01 % N	0·02 % N	0·05 % N
172,000	19	26	27	4
86,000	18	33	28	4
21,000	21	14	21	4
4,000	3	3	5	3
1,000	2	0	1	3
200	2	0	0	0

Results given in Table 5 show almost complete suppression of growth at 0·05 % N, even with a large inoculum. A similar optimum N concentration is shown as for asparagine, and so 0·02 % N was used in subsequent experiments.

It is probable that certain growth factors and other nutrients were carried over with the inoculum, since the spores were not washed, and dilution of the inoculum with corresponding dilution of these growth factors may have partly accounted for the decreased growth at low inoculum levels. However, this does not explain why this effect should have been most pronounced at the highest N concentration. It was thought that this phenomenon might be associated with the formation of toxic substances during autoclaving of the media, these substances possibly having a greater effect on a small number of spores. Compounds formed by the Maillard 'browning' reaction (Maillard, 1912) between amino acids and sugars have been shown to influence the metabolism of various micro-organisms. McKeen (1956) reported that such a substance was toxic to *Phytophthora fragariae* and other *Phytophthora* spp. A series of experiments was therefore designed to ascertain whether the behaviour of *P. infestans* was similar.

Effect of substances formed during autoclaving of the media

Indirect evidence on the formation of toxic substances during autoclaving of the media was provided by an experiment in which the medium (basal medium with asparagine at 0·02 % N) was autoclaved at 5, 10, or 15 lb. pressure for times ranging from 0 min. (gas turned off immediately the desired pressure was reached) to 20 min. The experiment was then repeated at a higher inoculum level, and the results of both experiments are shown in Table 6.

Table 6. *Effect on mycelial dry weight of* Phytophthora infestans *of time and pressure of autoclaving of basal medium + asparagine*

Pressure and time of autoclaving	Mycelial dry weight (mg.) Sporangia per 25 ml. culture solution	
	25,000	66,000
5 lb., 0 min.	47	65
10 lb., 0 min.	46	66
10 lb., 5 min.	41	60
10 lb., 10 min.	36	57
10 lb., 20 min.	28	47
15 lb., 20 min.	0	34

While an increase in the time and pressure of autoclaving the medium seems to have little effect up to 10 min. at 10 lb., above this level there is a drop in the final dry weight attained.

Growth was slightly greater in solutions which had been sterilized by steaming than in those autoclaved at 10 lb. for 10 min., but the increase was not thought great enough to outweigh the practical disadvantages involved in sterilizing large numbers of flasks by steaming. It was not found possible to grow *P. infestans* in Seitz filtered media, even when the pH of the medium, which rose considerably during filtration, was adjusted to 5·5 after sterilization. It was therefore decided that for practical purposes it was satisfactory to autoclave the media at 10 lb. for 5 min.

A series of experiments was then carried out in which all or part of the asparagine and glucose in the medium was previously autoclaved in more concentrated solution for 20 min. at 20 lb. The concentrated solutions contained 0·25 % N and/or 20 % glucose and if diluted ten times with the basal medium minus glucose gave concentrations of N and glucose of the same order as those used in previous experiments (0·025 % N and 2·0 % glucose, instead of 0·02 % N and 2·5 % glucose).

Three series of solutions were set up containing (*a*) an autoclaved mixture of asparagine and glucose, (*b*) an autoclaved glucose solution, and (*c*) an autoclaved asparagine solution. In each series proportions of the total N and/or glucose in the medium ranging from 1 to 100 % came from the autoclaved solutions. The control contained unautoclaved asparagine and glucose. The complete media were then re-autoclaved at 10 lb. for 5 min. The inoculum was equivalent to *c.* 29,000 sporangia/25 ml.

There is some indication that when all the glucose had been previously autoclaved growth was slightly decreased, but in general separate autoclaving of the asparagine and glucose beforehand seems to have had no significant effect on the amount of growth. However, when 50 % or more of the total asparagine and glucose came from the concentrated autoclaved mixture there was a marked decrease in growth. It therefore seems reasonable to suppose that this inhibition is due to a substance formed by the interaction of asparagine and glucose during autoclaving, and not simply to the breakdown of an essential group in the asparagine molecule.

Table 7. *Effect on the mycelial dry weight of* Phytophthora infestans *of various proportions of solutions of glucose, asparagine, and a mixture of these autoclaved before addition to basal medium*

	Mycelial dry weight (mg.)		
% total N and/or glucose previously autoclaved	Asparagine and glucose, autoclaved together	Glucose only, autoclaved	Asparagine only, autoclaved
100	5	30	36
50	18	46	42
20	43	50	38
10	37	52	40
1	46	43	41
0	44	—	—

Even in the presence of 0·25 % yeast extract, growth was substantially reduced when 50 % or more of the total asparagine and glucose had been previously autoclaved. It was only one-eighth of that in the control when all the asparagine and glucose had been previously autoclaved.

The autoclaved mixture, which was yellowish brown in colour, was no longer toxic after decolorizing with activated charcoal, but it is not known whether the toxic substance and the colouring matter are one and the same.

Since the effect of dilution of the inoculum was greater at a higher N concentration, which was tentatively ascribed to the presence of a greater amount of toxic substance in the medium, it was decided to re-investigate the effect of autoclaved asparagine and glucose at three inoculum levels (Table 8).

There seems to be some indication in Table 8 that the inhibitory effect of the autoclaved solution is greater when the inoculum is smaller, but the differences are too small to be conclusive.

Comparison of a series of amino acids

In this experiment, growth of three isolates of *P. infestans* on different N sources was investigated. The isolates were of Race 2,4, Race 1,4 and Race 4. The N sources were asparagine, aspartic acid, glutamine, glutamic acid, alanine, arginine and glycine, each at a total N concentration of 0·02 %. The 'complete medium' used for growing single spore cultures

Table 8. *Effect on mycelial dry weight of* Phytophthora infestans *of concentration of toxic autoclaved solution of asparagine and glucose at different inoculum levels*

% total N and glucose previously autoclaved	Mycelial dry weight (mg.) Approx. no. sporangia per 25 ml. culture solution		
	26,000	13,000	7000
100	8	6	5
50	28	26	21
20	37	34	33
10	35	34	31
1	37	38	30
0	35	30	36
Total	180	168	156

(see above, p. 16) was also used. The experiment was carried out using a large and a small inoculum. The spore concentrations of the three isolates were not exactly comparable but were of the same order. The results are shown in Table 9.

Table 9. *Mycelial dry weight (mg.) of* Phytophthora infestans *on basal medium with different amino acids and amides*

	Large inoculum*			Small inoculum†		
Nitrogen source	Race 2, 4	Race 1, 4	Race 4	Race 2, 4	Race 1, 4	Race 4
Asparagine	58	63	54	47	60	68
Aspartic acid	5	3	5	0	5	0
Glutamine	32	33	34	17	36	41
Glutamic acid	23	41	35	22	41	36
Alanine	14	51	36	4	1	42
Arginine	35	39	40	32	32	39
Glycine	5	38	11	2	0	23
'Complete medium'		Not included		124	126	171

*	Race 2, 4	56,000	Sporangia + zoospores per 25 ml. culture solution
	Race 1, 4	58,000	
	Race 4	50,000	
†	Race 2, 4	13,000	Sporangia + zoospores per 25 ml. culture solution
	Race 1, 4	7,000	
	Race 4	18,000	

In the cases of asparagine, aspartic acid, glutamine and arginine there was very little difference in the amount of growth of the three isolates, except that the growth of Race 2,4 on asparagine and glutamine at the low inoculum level was slightly less than that of the other races; otherwise there was no marked effect of inoculum size. On glutamic acid the growth of Race 2,4 was again less than that of the other two races, and the inoculum size had apparently no effect. However, growth on alanine and glycine did show differences at the two inoculum levels. On alanine Race 4 grew well at both inoculum levels, but Races 1,4 and 2,4 only grew when the inoculum was large; even then the growth of Race 2,4 was rather poor. The results with glycine are somewhat anomalous, as Race 4 grew better in the low inoculum series and Race 1,4 in the high inoculum series. Race 2,4, which failed to grow on glycine in this experiment, did so to a slight extent on other occasions.

It appeared that all these amino acids produced toxic substances when autoclaved in concentrated solution with glucose, but it was not possible from the evidence available to relate the toxicity of the autoclaving product to the suitability of the individual amino acids as sole sources of N.

In order to obtain some idea of the amount of toxic substances formed during normal autoclaving of the media, an experiment was set up to discover whether growth was increased when the N source and the basal medium including glucose were autoclaved separately. The same series of amino acids was used as in the previous experiment. One series of media was made up and autoclaved at 10 lb. for 5 min. in the usual way. In the second series, solutions of the amino acids and of the basal medium, both at double the usual strength, were mixed after autoclaving at 10 lb. for 5 min. There was hardly any difference in pH between the solutions mixed before and after autoclaving. The inoculum was equivalent to *c.* 6000 sporangia/25 ml. culture solution (Table 10).

Table 10. *Effect on mycelial dry weight of* Phytophthora infestans *of separate autoclaving of the nitrogen component of the medium*

	Mycelial dry weight (mg.)	
Nitrogen source	N source autoclaved with basal medium	N source autoclaved separately
Asparagine	43	48
Aspartic acid	4	3
Glutamine	31	33
Glutamic acid	21	28
Alanine	19	17
Arginine	34	36
Glycine	21	31

The differences between the two series are all rather small, but, except with aspartic acid (where growth was negligible) and alanine, growth was slightly more when the N compound had been autoclaved separately. This probably indicates that the presence of a toxic compound of glucose and amino acids has some effect in the media usually used, but that this effect is small under the usual conditions.

Discussion

The experiments described above indicate several possible reasons for the sporadic failure of growth in liquid culture of the isolate of *Phytophthora infestans* here studied. One of the most important factors determining the dry weight of mycelium attained in a given time was the size of the inoculum used initially.

It has been shown that a substance which is formed by the interaction of glucose and amino acids during relatively prolonged autoclaving is able to suppress the growth of *P. infestans*. It is probable that this substance is formed in small amounts during normal autoclaving and that an increase of autoclaving time or of N concentration increases the amount of the toxic substance formed. A fairly high proportion of the autoclaved asparagine/glucose solution had to be added to the medium to cause a marked reduction in growth, and it is difficult to believe that variation in the amount of the toxic substance caused by accidental variation in autoclaving time could be large enough to affect growth to any significant extent, at least at 0·02 % N. This is also indicated by the results of the experiment in which the N source was autoclaved apart from the rest of the medium, which procedure caused only a slight increase in growth.

There was evidently some minimum inoculum size necessary for growth to be established, and this size varied with the medium. For example, in basal medium with asparagine equivalent to 0·005 % N it was as low as 1000 spores/25 ml., while at 0·05 % N some 25,000 spores were needed. It seems likely that this phenomenon is associated in some way with the amount of toxic substance present in the solution, a larger number of spores being necessary for the establishment of growth at higher concentrations of the toxic substance. Observations on percentage germination of sporangia of Race 2,4 in media containing increasing amounts of the autoclaved asparagine/glucose solution did not directly support this hypothesis, since none of the percentage germinations appeared to differ significantly from the control. However, experience in making single spore isolates has shown that a spore which has produced a germ tube may not necessarily grow any further. When spores were transferred at too early a stage (i.e. when only a simple germ tube had formed) from the agar plate on which they had germinated, further growth did not occur. Thus the percentage germination in any medium does not necessarily give an estimate of the potentiality of the inoculum for establishing growth in that medium.

It was concluded that the basal medium plus asparagine at 0·02 % N could be used as a 'minimal medium' in genetical work, provided that the method used for detecting nutritionally deficient mutants was one in which a mass inoculum from each survivor after irradiation could be tested, otherwise some mutants might be missed.

This work was carried out during the tenure of an Ethel Sargant Research Studentship from Girton College, Cambridge and received financial support from the Agricultural Research Council. My thanks are due to Dr M. Keay and Miss M. Kostrowicka for supplying isolates of *P. infestans*, and to Dr N. F. Robertson for much encouragement and advice.

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STUDIES ON *PUCCINIA POLYSORA* UNDERW.

II. A CONSIDERATION OF THE METHOD OF INTRODUCTION OF *P. POLYSORA* INTO AFRICA

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Examination of herbarium specimens of world collections of maize rust has shown that, before 1949, *Puccinia polysora*, the American corn rust, was confined to the Caribbean area. The appearance of this rust in West Africa in 1949 raised the question of the method of introduction, the only source of infection at that time being over 3000 miles distant and separated by the Atlantic Ocean. A consideration of available evidence indicates that the pathogen was introduced as viable uredospores carried by air transport. The subsequent spread and severity of the epiphytotic emphasize the importance of this introduction in relation to plant quarantine.

Introduction

Whenever a new epiphytotic breaks out the following questions arise. First, is it in fact caused by a new pathogen or by one which has been present previously and overlooked, and secondly, if it is a disease new to the affected area, how was it introduced?

Several workers have suggested that *P. polysora* was not introduced into West Africa in 1949, but was, in fact, overlooked and at that time suddenly assumed a more virulent form. *P. polysora* bears strong superficial resemblance to a second rust of *Zea mays*, *P. sorghi* (Cammack, 1955), and before 1949 this latter rust had been reported in twelve territories in the African Continent. After Cummins (1941) had identified *P. polysora* on *Z. mays* in America, the collections of maize rusts at the Commonwealth Mycological Institute were re-examined. These included all available specimens from Africa and in 1944 it was established that only *P. sorghi* was present in the African Continent. Immediately after the severe outbreak of rust in Sierra Leone in 1949 specimens collected in the Colony were identified as *P. polysora* (Deighton, 1951). Specimens collected the same year in Ghana were, without exception, *P. sorghi*, and *P. polysora* did not appear in that territory until the following year, 1950. All available evidence based on herbarium collections and field observations points to the introduction of the rust in or about 1949, and none supports the theory that the fungus was present previously as a weak parasite of maize. Some possible methods of entry of the rust into the African Continent are discussed.

Introduction by inoculum carried in wind currents

Wind is the principal means of dissemination of rusts both locally and over great distances. Schmidt (1925) estimated the absolute distance a uredospore could be carried in air currents to be 1100 km. This calculation was based on physical reasoning and had no supporting experimental work. A better theoretical approach with a considerable amount of experimental evidence is that of Sutton (1932) and this is fully discussed by Gregory (1945). Stepanov (1935) calculated the absolute limit of dissemination of uredospores of *P. triticina* as 1282 km. Naumov (1939) calculated the theoretical limit of *P. triticina* to be around 1200 km. Moulton (1942) has illustrated the large overland distances travelled by rust spores in his study of black stem rust of wheat, which is carried northwards each year from Texas to Canada.

On release from the source uredospores may be raised high in the atmosphere by thermal convection and frictional turbulence. Stakman, Henry, Curran & Christopher (1923) trapped wheat leaf rust uredospores at a height of 16,000–16,500 ft. Height attained is of importance in the distance travelled from the source by uredospores in wind currents. Ukkelberg (1933) has shown theoretically that a wheat leaf rust uredospore at 5000 ft. in a 30 m.p.h. wind could travel a horizontal distance of 1000 miles.

Little is known of the dispersal of fungus spores over large areas of water. Bisby (1935) and McCubbin (1944) have found spores present in small quantities over mid-Atlantic. Pady & Kapica (1955) exposed nutrient agar plates and silicone-coated slides at heights of 8000 and 9000 ft. respectively on two trans-Atlantic flights and found uredospores to be comparatively rare, occurring as single uredospores on four slides exposed over eastern Canada and one on a slide exposed in a tropical air mass over Iceland.

It is known that prior to 1949 *P. polysora* was confined to Central America and the West Indies. On the appearance of the rust in West Africa that year the source of infection was 3500 miles distant and separated by the Atlantic Ocean.

Introduction by seed-borne inoculum

The possibility of seed-borne infection, with special reference to wheat rust, received much attention from early workers and their theories have been reviewed by Levine (1919). Chester (1946) states that in severely rusted wheat crops the grain has been observed to be orange-tinted by the heavy covering of uredospores. Naumov (1939) maintains that it is unlikely that uredospores carried on grains would be capable of initiating infection of the young seedling since the exposed tissues (coleoptiles, roots, etc.) are not susceptible and, by the time parts of the seedling prone to infection become available, the spores have lost their viability or germinated in the soil. Weber (1922) germinated maize and allowed the seedlings to grow until the radicle and coleoptile were approximately 0·5 in. long and then immersed them in an aqueous suspension of uredospores of *P. sorghi* for 5 min. The seedlings were then planted 2 in. deep and 4 weeks later mature uredosori were found 1·5 in. below the surface of the soil. The mechanical effect of rain splash could cause these sori to become exposed and afford a source of inoculum. There have been no reports of possible seed-borne transmission of *P. polysora*, but Rhind (1952) has suggested the possibility of uredospores becoming lodged in the rudimentary glumes.

Several experiments were designed to investigate the possibility of seed-borne infection. These were carried out in a greenhouse at Ibadan using the rust susceptible cultivar of maize, Lagos White. Since all experiments gave negative results, only a brief description of the methods is given.

I. *The immersion of maize grains in a uredospore suspension*

(*a*) Maize grains were washed in running water for 15 min. and then immersed in a thick suspension of uredospores in distilled water for 5, 15 and 60 min., and 12 hr. respectively. The batches of grain were removed from the suspension with forceps and planted 1 in. deep in sterilized sand culture.

(*b*) Previously washed grains were soaked in sterile water for 24 hr. and then immersed in a uredospore suspension for the same range of times as in I*a*, before being planted in sand culture.

II. *The inoculation of the surface of maize grains*

(*a*) Dry grains were rapidly washed with 70% alcohol, rinsed with sterile water and allowed to dry thoroughly before being heavily dusted with uredospores. Half the grains were planted 1 in. deep and the rest 2 in. deep in sand culture with forceps.

(*b*) Grains were washed in running water for 15 min., rinsed in sterile water and then dipped in warm 1% agar. The surface of the agar was dusted with uredospores and the grains were then planted as in II*a*.

III. *A repeat of the experiment by Weber* (1922)

(*a*) Maize grains were germinated until the coleoptiles were 0·5 in. long. The seedlings were then immersed in a uredospore suspension for 5, 15 and 60 min. and planted 2 in. deep in sand culture.

(*b*) Washed grains were soaked in sterile water until the coleoptiles began to grow. The seedlings were then dipped in warm 1% agar, dusted with uredospores and planted as previously.

The potted seedlings from the above experiments were kept in a greenhouse for 5 weeks and examined every 2 days. All experiments gave negative results.

Meijers (1938) observed that *P. sorghi* was unknown in Holland until a North American variety of maize was imported for experimental purposes. *P. sorghi* appeared on the first generation of this variety and subsequently on local varieties. Humphrey & Cromwell (1930) suggest that the introduction of *P. glumarum* into Argentina may have been associated with the importation of grain. Naumov (1939) mentions that the possibility of inter-continental spread of rust becomes more likely when one considers the many years of grain commerce between the continents, and that it only requires a single successful spore in the correct environment to establish the disease.

In the immediate post-war years there were large imports of bulk maize grain into West Africa from the Americas and especially into Ghana, for use both as fodder and seed.

INTRODUCTION ON A LIVING HOST

Records of grass importations kept by the Nigerian Federal Department of Agricultural Research reveal that clonal material of *Tripsacum laxum*, an alternative host of *P. polysora*, was imported into Nigeria from Trinidad in 1945 and found to be infected with that rust. The clonal material was intercepted by the Plant Quarantine Division and all infected plants destroyed, the remainder being sterilized and subsequently multiplied. Although a most careful watch was kept no further infection was observed.

INTRODUCTION OF INOCULUM BY AIR TRANSPORT

Chester (1946) states that modern inter-continental air transport could be a most effective means of introducing rusts. The rapidity of travel would ensure that, if an aeroplane or its contents were contaminated, the uredospores would have an excellent chance of being liberated at the point of arrival in a viable state.

During the war and immediate post-war years foodstuffs including green vegetables were flown into West Africa from the Americas to supply forces stationed on the coast. Among these fresh vegetables was sweet corn 'on the cob'. On frequent occasions uredosori of *P. polysora* have been observed growing on the inner surfaces of the husks of maize ears. Samples of maize grain, for experimental purposes, were also frequently flown in during the post-war years.

DISCUSSION

Most of the work on the limits of spore dispersal has been based on physical reasoning with no experimental backing. The very large number of spores expected to be released from the nearly infinite number of points at the origin of the rust, Central America and the West Indies, would have no absolute theoretical limit of dispersal. The chances are that spores often complete the 3500 miles journey, but are almost certainly non-viable on arrival. Experiments carried out at Ibadan have shown that the uredospores of *Puccinia polysora* failed to germinate after exposure to 5° C. for longer than 12 hr. At some stage of the journey over mid-Atlantic the spores would without doubt be subjected to similar, and even lower, temperatures for long periods.

Present opinion tends to discount wind as a medium of dispersal of fungus spores over large areas of ocean and this is supported by present-day species isolation throughout the world. Presumably *P. polysora* was in America for many years prior to its discovery in 1897 and in all the years that maize has been present in the African continent the rust has failed to establish itself there by normal processes.

Experiments on the inoculation of grain all gave negative results. These experiments, however, were not sufficiently comprehensive and, owing to the difficulty of simulating natural conditions of seed contamination experimentally, are far from being conclusive. The possibility of introduction by spores adhering to grain imported by a sea-route would be greater in rusts in which the viability of the uredospore was longer. *P. polysora*, in laboratory tests at Ibadan, showed a reduction of viability of 50 % after 27 days and total death after 50 days. The chances of survival during a long sea voyage are small. The observations of Meijers (1938) and Humphrey & Cromwell (1930) indicate that the possibility of introduction by this method cannot be discounted completely.

It is unlikely that the rust was introduced on infected *Tripsacum laxum* in 1945. The remaining original material which was not destroyed was multiplied and distributed throughout Nigeria and, in the years between its introduction and the appearance of *P. polysora* on *Zea mays* in Nigeria, no rust was observed on the *T. laxum*, nor on *Z. mays*. The imported *T. laxum* was clonal material from Trinidad, in which area it is commonly infected with a rust currently classified as *P. polysora*. Cross-inoculation studies at Ibadan have repeatedly failed to produce infection on *T. laxum* with inoculum obtained from infected *Z. mays*, and it is possible that the respective strains are incompatible on the two hosts. No similar cross-inoculations have been done in Trinidad to support this theory.

On the basis of existing evidence, it is most likely that the rust was introduced by air-transport. Nevertheless, this must remain a supposition, because the possible methods of introduction cannot be reproduced experimentally. The problem is, however, one of great interest with respect to plant quarantine, since not only is the rust now present throughout all tropical maize-growing areas, but disease saturation has occurred.

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CERCOSEPTORIA CYDONIAE SP.NOV. ON QUINCE (*CYDONIA VULGARIS* L.) IN IRAN

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(With Plate 1 and 2 Text-figures)

Cercoseptoria cydoniae sp.nov. causing angular brown leaf spots on quince from Iran is described. Within the spots thin, hyaline, septate hyphae fill the space between the upper and lower epidermis and produce sporodochium-like structures (35–130 μ diameter) under the upper cuticle, and small mycelial knots under the lower epidermis at the stomata. When moist the sporodochia produce either short thin hyaline conidiophores (5–22 × 0·7–1 μ) each bearing a single septate or non-septate *Cercosporella*-like conidium at the tip, or else scattered or entwined hyphae on the side of which spores are produced singly, on very short sterigmata. Spores are 5–119 × 1·3–2·5 μ with 0–11 septa. The fungus grows very slowly in culture. Colonies are cerebriform bacteria-like on some media and slightly woolly on other media. Spores are produced abundantly in cultures where every cell of a hypha or of an existing spore may produce a short sterigma bearing a single conidium.

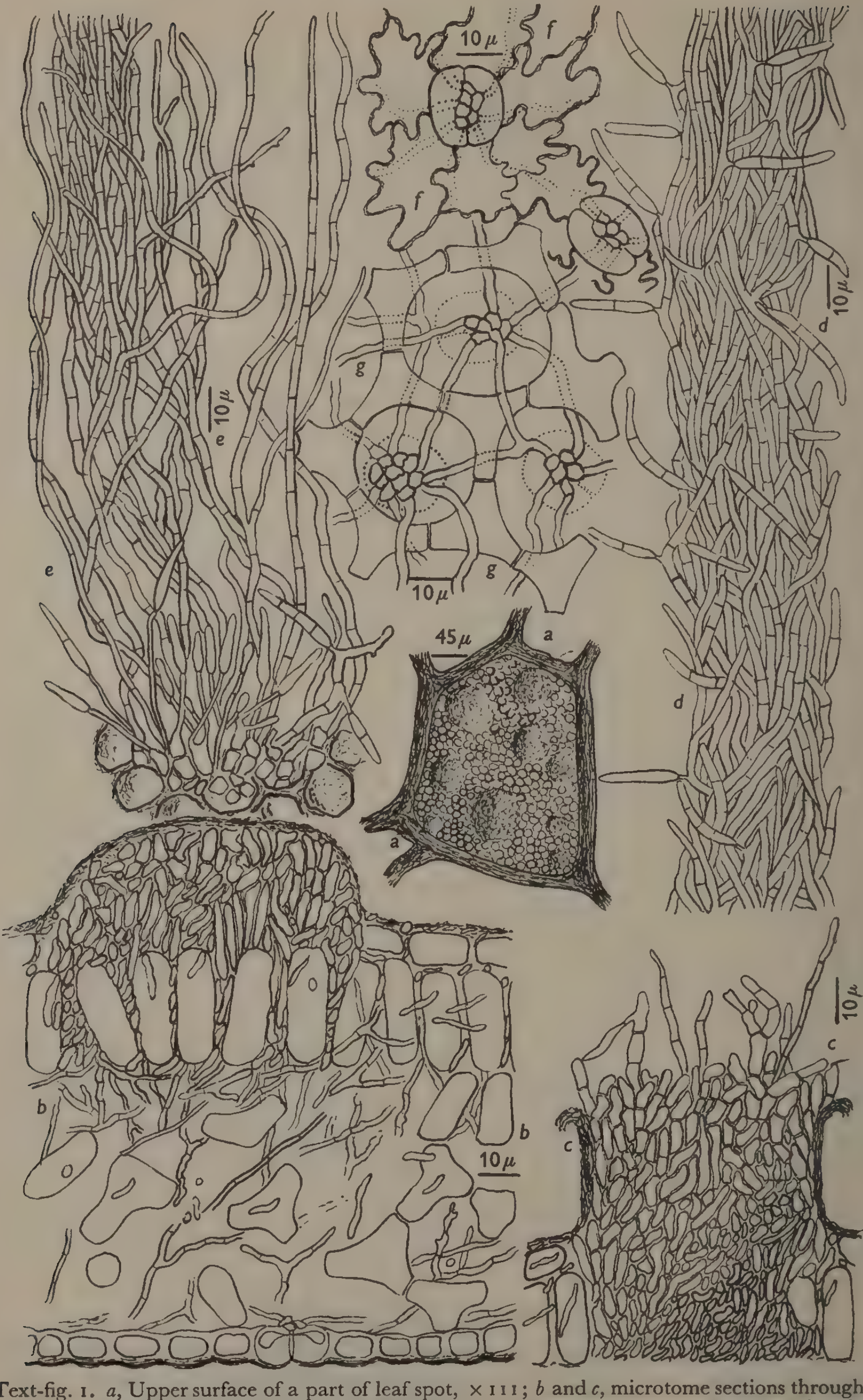
Cercoseptoria cydoniae sp.nov. (Pl. 1, Text-figs. 1, 2)

Maculae amphigeneae, angulosae, venis folii limitatae, superne atrobrunneae, inferne pallidiores, 1–5 mm. diam., vel confluendo majores. *Mycelium immersum* ex hyphis ramosis, septatis, hyalinis 0·7–3·5 (1·5–2·0) μ compositum; hypophyllum reticulatum, in cameris substomatalibus nodosum; idemque epiphyllum hypostromata sporodochialia formans. *Hypostromata* sparsa vel gregaria, in vel sub epidermide oriunda, primo infra cuticulam integram nidulantia, dein ad 15–35 μ alta erumpentia, basim versus per cellulas folii prismaticas penetrantia, et eas denique obliterantia, 30–130 (50–80) μ diam.; in siccitate diu sterilia, sed in humiditate continua mox in hyphas fertiles vel steriles, simplices vel funiculosas excrescentia, vel rarius in conidiophora generis typicalia, et ita haec in 'sporodochia', illa in 'synnemata' transeuntia. *Conidiophora*, sic appellata in descriptionibus Cercoseptoriae, simplicia, erecta, gracilia, bacilliformia hyalina, 5–22 μ longa, 0·7–1·0 μ lata, in superficie sporodochii oriunda, et conidia apiculariter singulatim gerentia. *Hyphae fertiles secundariae*, sterigmatibus lateralibus, minutis, cylindricis, 2 μ altis, sine ordine sparsim praeditae, et in his conidia singulatim gerentes; aliquando unae cum hyphis sterilibus in synnemata laxa vel compacta, cylindrica, ad 1 mm. alta, 15–45 μ lata coalescentes. *Conidia* praecipue ad scolecosporas pertinentia et obclavata, ex cellula basali vel ejus propinquitate ad apicem obtusum gradatim angustiora, basi acuta, rarius anguste fusiformia, gracilia, farinosa, recta vel curvata, etiam flexuosa, hyalina, omnino 0–11 septata sed primo ad septa non vel vix constricta, 5–119 μ longa, 1·3–2·5 μ lata, saepius ad 4-septata, 15–45 μ longa; interdum per sterigmata conidia secundaria similia gerentia.

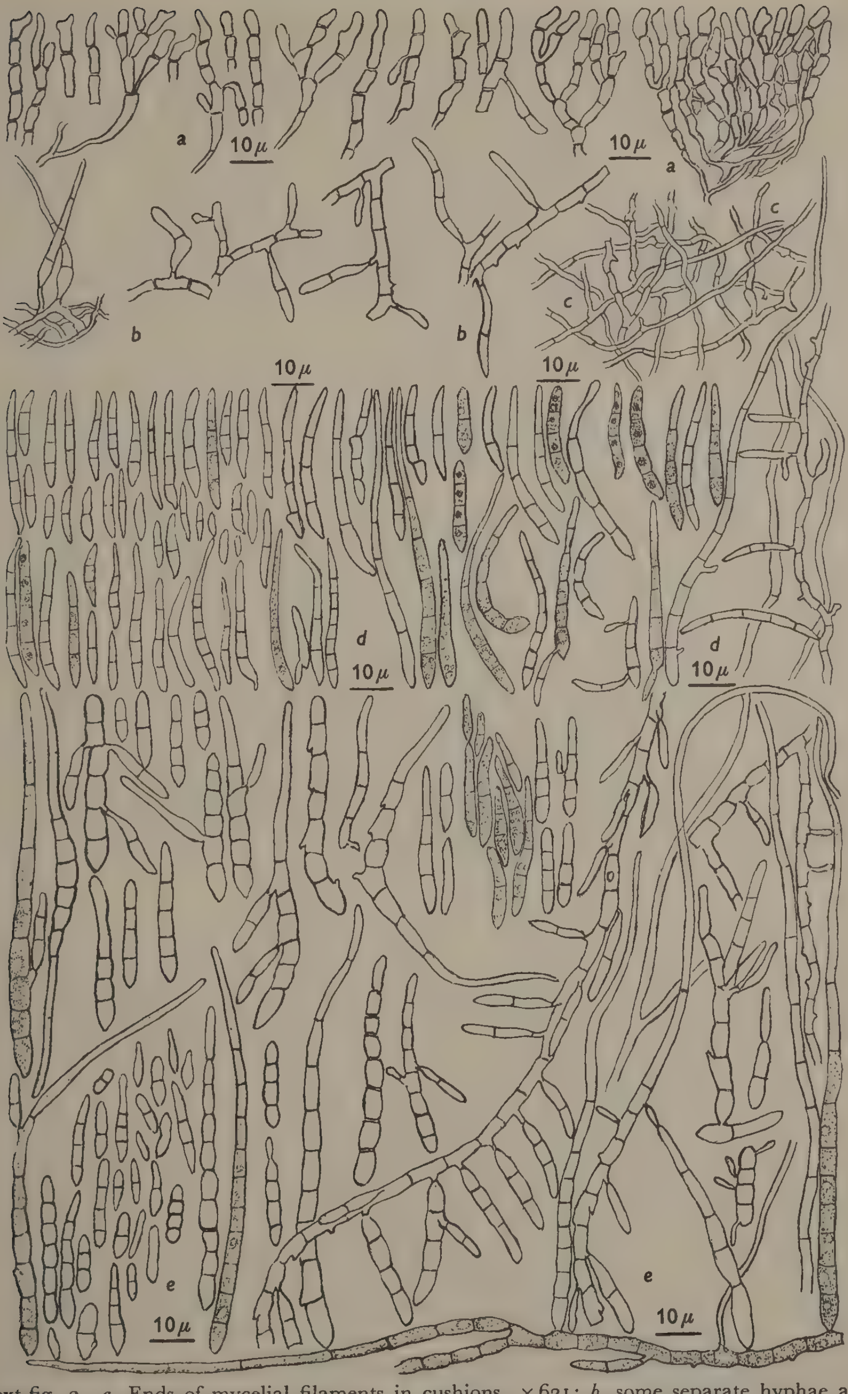
Hab. in foliis vivis *Cydoniae vulgaris* L.; Dezfool, Iraniae austro-occidentalis; 19 Dec. 1956 (leg. Mir Salavatian). Typus: Herb. I.M.I. no. 69900.

MATERIALS AND METHODS

To isolate the causal agent, four infected spots were removed from the leaf, immersed in 1/1000 mercuric chloride solution for about 1 min., washed with sterile water and each put in the middle of Petri dishes: two



Text-fig. 1. *a*, Upper surface of a part of leaf spot, × 111; *b* and *c*, microtome sections through a diseased spot, × 621; *d*, part of mycelial cord with spores grown from a cushion when leaf spot has been under moist conditions, × 621; *e*, conidiophores with conidia and mycelial filaments grown from a cushion, × 621; *f*, lower epidermis with reticulate mycelium under it, × 621; *g*, hypodermal cells and reticulate mycelium, × 621.



Text-fig. 2. *a*, Ends of mycelial filaments in cushions, × 621; *b*, some separate hyphae and conidia from leaf spot, × 621; *c*, internal reticulate mycelium, × 621; *d*, conidia and hyphae in nature, × 621; *e*, conidia and hyphae from a culture on potato-dextrose, × 621.

spots of wheat flour agar (40–20, in 1000 water = WFA) and two of glucose peptone sodium chloride agar (10–20–5–15, in 1000 water = GPSA). After a week the procedure was repeated.

One of these eight leaf spots gave a pure culture of *Cladosporium*, one *Cephalosporium*, four a mixture of *Cladosporium* and *Cephalosporium*. No growth was seen in one GPSA plate of the first isolation and in one WFA plate of the second isolation until at about 2 weeks when a very slight growth about 1 mm. diameter was seen round the edge of the spots. Large numbers of *Cercosporella*-like spores were produced in both plates. As the growth in these two plates was very slow the leaf spots were removed and placed in the middle of two other Petri dishes with fresh media to provide more moisture. Here again the growth was very slow. The maximum size of the colony on WFA was 27 × 23 mm. and on GPSA 15 × 12 mm. (Pl. 1, fig. 6) which included the diameter of the leaf spots (about 4 mm.). Any growth of this *Cercosporella*-like fungus in the other six plates could have been checked at an early stage by the faster growth of the *Cladosporium* and *Cephalosporium*.

A few spores and conidiophores of *Cladosporium* were occasionally seen on the leaf spots. The coloured hyphae of *Cladosporium* were very different from the thin mycelial filaments which occupied the parenchyma of the leaf and it was unlikely that this *Cladosporium* was the cause of the disease.

It appeared probable that the *Cercosporella*-like fungus was the causal agent. An attempt was made to produce spores by putting leaf spots on moist filter-paper in a sterile Petri dish. After 3 days long columns of roughly interwoven hyphae were visible on every cushion on the upper surface of the leaf spots. Some parts of the mycelium produced large numbers of spores similar to those of the *Cercosporella*-like isolations. No *Cladosporium*, *Cephalosporium*, or any other impurity was seen on these spots.

Description of the fungus

On the leaf surface the fungus produces angular amphigenous spots (sharply limited by small veins) 1–5 mm. diam., but when confluent they may form larger spots the borders of which always remain irregular. The upper surface of the spot is homogenous dark brown while the lower surface is a somewhat lighter brown (Pl. 1, fig. 1). No special colours or reactions are present at the borders of the spots.

The mycelium is composed of very thin, hyaline, septate hyphae, 0·7–3·5 (usually 1·5–2) μ wide (Text-fig. 2*c*). It occupies and disorganizes the mesophyll. It is looser near the lower epidermis, under which it forms a reticulum. Here the hyphae converging towards stomata form a small mycelial knot just under each aperture (Text-fig. 1*f*, *g*). Microscopic examination of a dissected diseased spot shows the reticulate mycelium clearly. The mycelium is denser near the upper epidermis where the hyphae, dichotomizing several times, end in somewhat thicker, closely septate branches. These form compact sporodochium-like masses under the upper cuticle.

Traces of hyphae may be present in the upper epidermis and cuticle.

With age the ends of filaments that form cushions or sporodochia may become disorganized and form separate fragments which usually retain their angular ends (Text-fig. 2*a*).

Under low magnification the cushions are seen as small white circles scattered over the spot on the upper surface of the leaf. They are mostly separate, but sometimes two or three become joined together. Addition of lactophenol cotton blue shows up the cushions more clearly (Text-fig. 1*a*).

Under dry conditions the cushions cease to grow beneath the upper cuticle. They measure 35–130 (usually 50–80) μ diam. and rise about 15–35 μ above the epidermis. Internally they occupy the parenchyma of the leaf down to the lower ends of the palisade cells (Pl. 1, fig. 2; Text-fig. 1*b*, *c*).

Under moist conditions the distal ends of filaments which form the cushion develop one or two short cylindrical, hyaline, conidiophore-like appendages on the tip of each of which is produced a small conidium. These conidia are usually unicellular but may have one or more septa. The conidiophores are 5–22 × 0·7–1 μ (Text-fig. 1*e*). When growth is more luxurious, long, slender, hyaline, septate hyphae are produced from the distal ends of cushion filaments. They are sterile or fertile and may be very loose and scattered or more or less entwined, forming coremium-like mycelial cords up to about 1 mm. high and 15–45 μ wide. The hyphae may be twisted together but are not fused (Text-fig. 1*d*, *e*; Pl. 1, fig. 7). Hyphae were not seen to emerge from the lower surface of the leaf.

Conidia are occasionally formed in small numbers under dry conditions. They are produced in all directions from the sides or tips of short outgrowths from the ends of filaments in cushions and on the side of internal hyphae exposed to the surface by minute cracks in the cuticle or epidermis. Under moist conditions the production of conidia is considerably increased. They are usually formed on the tips of very short sterigmata which may grow up from every cell of the hyphae and from cells—especially the lower cells—of previously formed conidia. No true conidiophores are produced from aerial mycelium. The sterigmata are not usually longer than 2 μ, but may be up to 4 μ.

The conidia are *Cercosporella*-like, hyaline, slender, straight or curved, sometimes flexuous and occasionally fusiform. When septate they are occasionally constricted at the septa. The basal cell is usually wider, more or less pointed at its insertion and tapering towards the apex. The cell contents are usually homogenous but sometimes granular with oil droplets. Conidia are 5–119 μ long and have 0–11 septa, but are usually less than 45 μ with up to 4 septa. On diseased spots which had been in a moist chamber for 3 days about 5 % of conidia were without a septum and usually up to 13 μ long; about 50 % had 1 septum and were usually 9·5–19·5 μ; about 25 % had 2 septa and were usually 14–23 μ; about 22 % had 3 septa and were 16–32 μ; about 3 % had more than 3 septa and less than 1 % had 7 septa. The width of conidia varied much less—1·3–2·5 (usually 1·6–2·2) μ (Text-fig. 2*d*).

In culture. The growth of the fungus is very slow in artificial media. The largest colony produced from a 5 mm. diam. inoculum in a Petri dish of WFA medium was about 25 mm. diam. and on GPSA almost 16 mm.

(Pl. 1, fig. 6). On slopes a streak did not grow more than about 7 mm. wide and never occupied the whole surface of the slope (Pl. 1, fig. 3). On some media such as GPSA (Pl. 1, fig. 5) and tryptone sodium sulphite iron citrate agar (10–1–0·5–20, in 1000 water = TSIA) the colonies are bacteria-like with cerebriform wrinkles. On other media such as WFA, potato dextrose and glucose peptone agar (20–10–20, in 1000 water = GPA = Langeron medium) growth is at first bacteria-like and with cerebriform contour, but it ultimately assumes a woolly appearance (Pl. 1, fig. 4).

Table 1. *Growth of* Cercoseptoria cydoniae *on five media*

(Inoculation was made in the centre of each plate. For explanation see text.)

Media	Age of culture	Contour of colony	Margin of colony	Colour of colony	Size of colony	Change or not in medium
TSIA	10 days dry	Cerebriform; bacteria-like	Finely denticulate; irregular	Chestnut brown	4 mm. diam.; 2 mm. raised; 5 mm. diam.	Darkened
GPSA	10 days dry	Cerebriform; bacteria-like	Finely denticulate; irregular	Chestnut brown	5 mm. diam.; 3 mm. raised	Darkened
GPA	10 days dry	Centre: cerebriform; bacteria-like; around: woolly	Woolly; almost round	White; centre: grey; margin: white	5 mm. diam.; 1 mm. raised; 9 mm. diam.	No change
WFA	10 days dry	Not cerebriform, not bacteria-like; woolly; concentric circles	Woolly; round	White; centre: grey; margin: white	7 mm. diam.; not raised; 13 mm. diam.	No change
PDA	10 days dry	Cerebriform; bacteria-like; woolly	Crenulate; woolly, round	Grey; centre: grey; margin: white	8 mm. diam.; 3 mm. raised; 16 mm. diam.	No change

TSIA and GPSA, which have sodium sulphite and sodium chloride, respectively, as one of the ingredients, darken. Comparing GPSA with GPA, where the only significant difference is absence of sodium chloride in GPA associated with absence of darkening of the medium, it is thought that the presence of sodium in the medium may be a factor in this phenomenon.

On the media used, mycelial growth was usually sparse, but spores were developed in large numbers. They were produced either directly, or on very short sterigmata, from almost every cell of the hyphae or from previously formed spores (Text-fig. 2*e*).

In WFA cultures sterile mycelial cords were sometimes present as in nature. No sporodochia or conidiophores were seen in cultures.

In cultures the mycelium is composed of hyaline, septate hyphae having granular contents and droplets. The hyphae are usually slightly wider than hyphae in nature, being $1{\cdot}3$–$4{\cdot}5\,\mu$ wide. The sterigmata are not longer than $2\,\mu$.

Conidia in cultures are similar to those produced in nature but the

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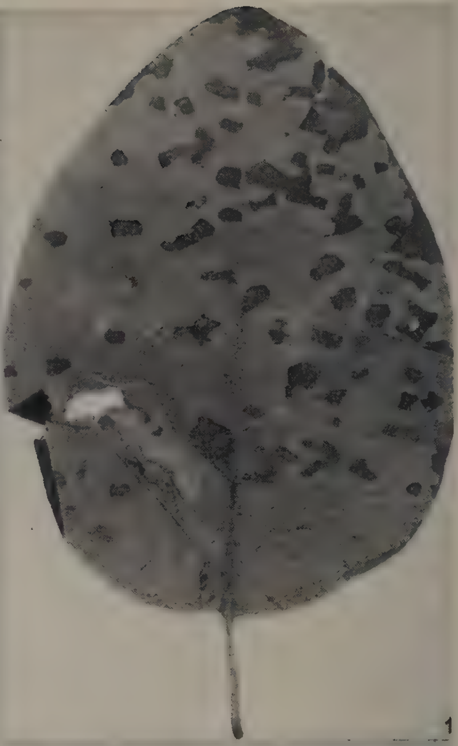
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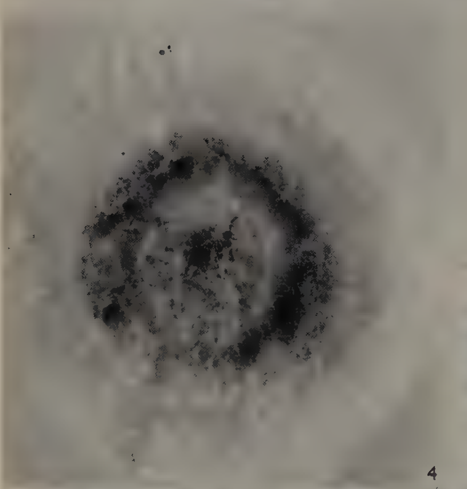
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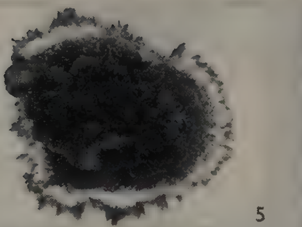
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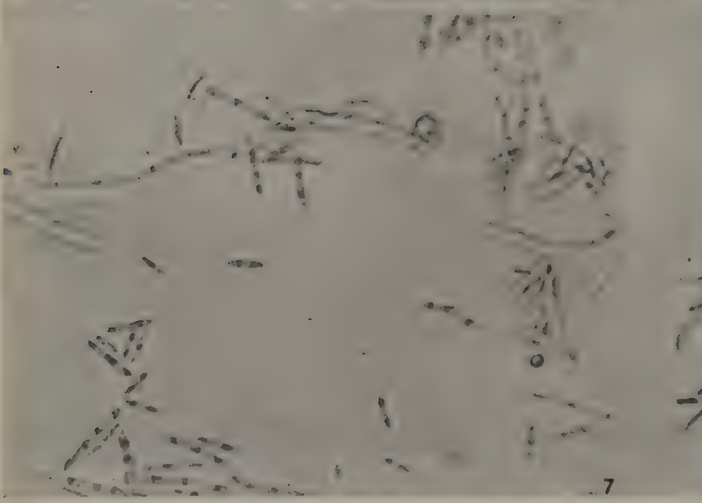
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base is sometimes less pointed or even hemiellipsoidal. The apices of conidia may also be less pointed and may even be rounded in short conidia. In long conidia the upper part is usually slender and filiform. Constriction at the septa is more frequent than in nature. Production of secondary conidia from cells of primary conidia is also more common than in nature and so is the production of branches from conidia, especially from the basal cell. The conidia are slightly larger and have more septa than in nature. They are 5–135 μ long with 0–12 septa, but usually up to 95 μ long with 1–7 septa. The width of conidia in cultures varies from 1·5–3·8 μ. Usually the basal cell is widest, but some conidia are cylindrical or fusiform (Pl. 1, fig. 8).

I wish to thank Prof. P. H. Gregory and Dr C. C. V. Batts of the Imperial College of Science and Technology for help in the preparation of this paper, and also Dr M. B. Ellis of the Commonwealth Mycological Institute for assistance with the classification of the fungus. I also thank Mr E. W. Mason for preparing the Latin diagnosis.

EXPLANATION OF PLATE 1

Cercoseptoria cydoniae on quince

Fig. 1. Lower surface of diseased leaf (natural size).

Fig. 2. Section through a disease spot showing a sporodochium on the upper surface of the leaf. × 480.

Fig. 3. Slope potato-dextrose, 18-day-old culture.

Fig. 4. Single colony in the middle of a plate on WFA medium, photographed when medium had been dried. × 6.

Fig. 5. Single colony in the middle of a plate on GPSA medium, photographed when medium had been dried. × 6.

Fig. 6. Maximum size of a colony begun from a surface sterilized leaf spot on GPSA medium, after 16 days removed to a new plate with fresh medium and photographed when medium had been dried. Natural size.

Fig. 7. Part of a mycelial cord bearing spores, grown from a cushion under moist conditions. × 480.

Fig. 8. Conidia and sterigmata from mycelial cells and from primary conidia on potato-dextrose agar. × 480.

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THE POLYPORACEAE OF THE MUSSOORIE HILLS (INDIA). IV

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(With 3 Text-figures)

Descriptions are given of *Polyporus indicus* Massee var. *depauperatus* var.nov., *P. minutisporus* sp.nov., and *Fomes annosus* (Fr.) Cooke var. *indicus* Wakef. apud Hole.

Under the leadership of Prof. P. N. Mehra an excursion is made every year from the Botany Department of the Panjab University to the Mussoorie Hills in the north-western Himalayas (2000–7000 ft.) to study the cryptogamic flora. The Polyporaceae are being investigated by Dr K. S. Thind and his students.

In the first three contributions (Thind & Chatrath, 1956, 1957; Chatrath & Bindra, 1957) twenty-three species and one new variety were described. This paper deals with one new species, one new variety, and a variety of *Fomes annosus* which has, hitherto, been overlooked. The fruit-bodies have been described from fresh specimens, supplemented with dried material and that preserved in alcohol formalin.

Type material of the new taxa has been deposited in the herbaria of the Panjab University and the Royal Botanic Gardens, Kew.

The numbers of the species are the serial numbers of the polypores of the Mussoorie Hills.

25. **Polyporus indicus** Massee var. **depauperatus** var.nov. (Fig. 1)

A typo differt sporis minoribus, (4–) 5–6 × (3·5–) 4–4·5 μ, cuticula absentia, dimidiata vel ligulata.

Habitat on the base of a living deciduous tree, Chakrata Road, Dehra Dun, India, 31 July 1953, **225** (Typus).

Hymenophore substipitate, solitary or sometimes caespitose with two or three pilei in a cluster, coriaceous when fresh, becoming hard and somewhat brittle on drying. *Pileus* dimidiate to ligulate, entire or lobed, 2·2–7 × 1·9–5·5 cm.; up to 0·6 cm. in thickness, narrowed behind into a short, lateral, yellowish brown stipe-like base, 0·7–2·2 cm. × up to 1·3 cm.; upper surface yellowish brown, radially and irregularly striate, with narrow chestnut or dark brown and broad yellowish brown concentric zones; cuticle absent; hymenial surface light chocolate brown, with sterile white or yellowish margin, 0·2–0·7 cm. wide. *Pores* angular, 3–4 per mm. *Tubes* in a single stratum, which is decurrent on the stipe-like portion of the pileus, up to 0·2 cm. in length. *Context* yellowish brown, cinnamon in dried specimens, blackening in potassium hydroxide solution, zoned, up to 1·5 cm in thickness. *Hyphal structure* monomitic. Generative hyphae yellowish brown, branched, septate, 4–8 μ diam., with thin or slightly thickened walls, and lacking clamp connexions. *Basidia* clavate, subhyaline, 4–6 μ diam., bearing four short sterigmata. *Spores* dark amber brown, in reality very broadly elliptical, but appearing almost ovate or subglobose, uniguttulate, (4–) 5–6 × (3·5–) 4–4·5 μ.

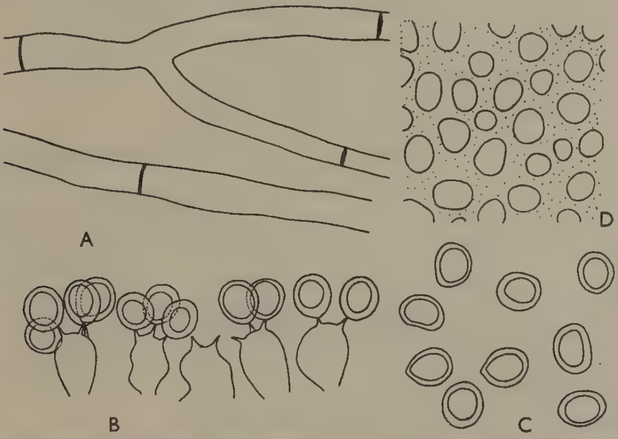

Fig. 1. *Polyporus indicus* var *depauperatus* var.nov. A. Hyphae, × 750. B. Basidia, × 1150. C. Basidiospores, × 1150. D. Pores, × 30.

P. indicus var. *depauperatus* differs from var. *indicus* in the small dimidiate or ligulate, substipitate hymenophores, absence of a cuticle, and in the smaller spores.

P. indicus var. *indicus* may form large centrally stipitate rosette-like clusters of imbricate pilei up to 30 cm. diam., as figured by Lloyd (1912, Fig. 461). The pilei possess a well-developed horny cuticular layer, except at the extreme actively growing yellowish margin, and have a dark purplish brown surface, ornamented with rather crowded concentrically sulcate ridges. In all the previous collections of this species preserved in the Kew Herbarium, whether from India or Malaya, the presence of a well-defined cuticle is a marked feature. The surface of the pileus is often described as glabrous, although it has a minutely velvety or pruinose appearance to the eye. The difference in the size and shape of the hymenophore, together with the absence of a cuticle and consequent difference in texture of the surface, renders var. *depauperatus* very unlike var. *indicus* in its gross morphology. However, the colour of the flesh and the hyphal structure are identical in each case. There is also close similarity in the contrast between the colour of the flesh and that of the tubes as seen in sections through the fruit-bodies. Further, there is very close agreement in the shape, colour, and size of the spores, although they are rather larger (5·5–7 × 5–6 μ) and often less distinctly elliptical in var. *indicus*.

After a detailed comparison between the present collection and the gatherings of *P. indicus* in the Kew Herbarium, there seems little doubt that the differences are insufficient to warrant the description of a new species.

26. **Polyporus minutisporus** sp.nov. (Fig. 2)

Hymenophorum solitarium vel imbricatum. *Pileus* dimidiatus, applanatus, sessilis, 1·5–6 × 2–5·5 × 0·4–1·5 cm.; superficie superiore luteo-brunnea, zonata, pubescenti; superficie inferiore fusco-cremea. *Pori* rotundati, 5–7 per mm. *Tubuli* usque ad 6 mm. longi. *Substantia* cremea, 1–5 mm. crassa. *Basidia* clavata, minuta, quadrispora. *Sporae* minutissimae, hyalinae, ellipsoideae, uniguttulatae, (2–) 2·5–3·5 × 1–1·5 μ.

Habitat on stump of a deciduous tree, the park, Mussoorie, India, 11 Sept. 1953, **226** (Typus).

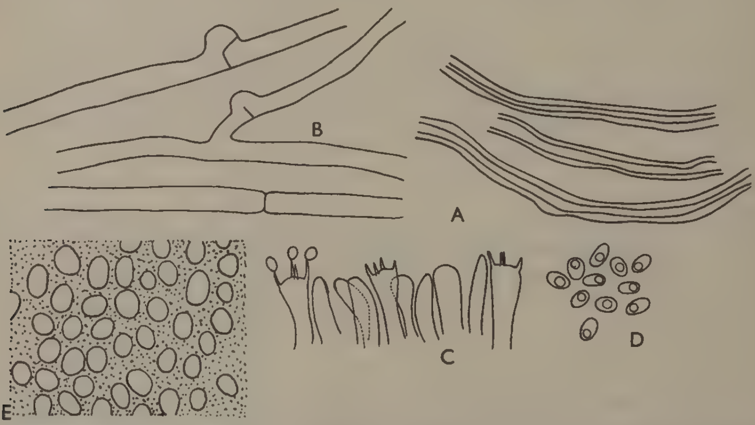

Fig. 2. *Polyporus minutisporus* sp.nov. A. Skeletal hyphae, × 750. B. Generative hyphae with clamps, × 750. C. Basidia, × 1150. D. Basidiospores with a small guttule, × 1150. E. Pores, × 25.

Hymenophores annual, solitary or imbricate, fleshy, tough and brittle when fresh, quite hard and woody on drying. *Pileus* dimidiate, applanate, sessile, 1·5–6 × 2–6·5 × 0·4–1·5 cm.; upper surface yellowish brown, darkening on drying, concentrically zonate, pubescent, lacking a cuticle; hymenial surface deep cream coloured, darkening on drying, with a sterile margin, 1–4 mm. wide. *Pores* rounded, 5–7 per mm., dissepiments not toothed. *Tubes* in a single stratum, up to 6 mm. in length. *Context* cream coloured, 1–5 mm. thick. *Hyphal structure* difficult to interpret. Two of us (K. S. T., M. S. C.) regard the structure as dimitic, whilst the other (D. A. R.) would prefer to see further gatherings before committing himself. There are thin-walled, hyaline, branched hyphae, 2·5–5 μ diam., with prominent clamp connexions at the septa. Sometimes, however, segments up to 650 μ in length may become thick-walled. In addition, there are very long, hyaline, pseudoseptate, branched or unbranched hyphae, 2·5–5 μ diam., with very thick walls, in which the lumen is almost obliterated. These hyphae originate from the thin-walled elements to which they are joined by a clamp connexion, and extend for long distances (e.g. 1000 μ), often without branching. They are thick-walled at their point of origin, but become thin-walled toward their apices. One of us (D. A. R.) has been unable to trace these elements to their free extremity, and since segments of the thin-walled hyphae may become thick-walled the exact interpretation of the hyphal construction is uncertain. *Basidia* clavate, minute, with four sterigmata. *Spores* very minute, hyaline, elliptical, uniguttulate, (2–) 2·5–3·5 × 1–1·5 μ.

27. **Fomes annosus** (Fr.) Cooke var. **indicus** Wakef. apud Hole in *Indian For.* **53**, 437, 1927 (Fig. 3).

A typo differt poris minoribus, 4–5 per mm.
Habitat on spruce (*Picea morinda*), Jaunsar, U.P., coll. R. S. Hole, 7 Oct. 1922 [lectotype].

Hymenophore perennial, resupinate or pileate, tough and coriaceous when fresh, hard and woody on drying. *Pilei* applanate, often imbricate, 1·5–9 × 4–19 × 0·5–2·0 cm., surface brown, previous years growth turning dark brown to almost black, uneven, rough, concentrically sulcate, not cracking, cuticle present, surface minutely pubescent. Margin acute, white, changing to brown on drying. *Hymenial surface* plane or convex, cream coloured, with sterile white marginal zone 0·5–1·5 mm. wide, becoming brown on drying. *Pores* round to oval or somewhat irregular, 4–5 per mm. *Tubes* usually stratified, up to 6 mm. in length, eventually becoming filled with hyphae. *Context* cream coloured, 2–7 mm. thick, delimited above by a hard black horny cuticle of agglutinated hyphae with brown contents. *Hyphal structure* dimitic. Skeletal hyphae 2·5–4·5 (–5) μ diam., very elongate, subhyaline, aseptate, unbranched, with strongly thickened walls (0·4–1·6 μ), which completely obliterate the lumina. The pubescence of the pileus appears to be formed of the terminations of skeletal hyphae which protrude through the cuticle. Generative hyphae 2–4 μ diam., branched, hyaline, thin-walled, lacking clamp connexions at the septa. *Basidia* 10–15 × 4–6 μ, with four straight or curved sterigmata 1·6–2·4 μ in length. *Spores* smooth, hyaline, nonamyloid, monoguttulate, broadly elliptical to subglobose (3·5–) 4–5 × 3–4 μ, with a small lateral apiculus.

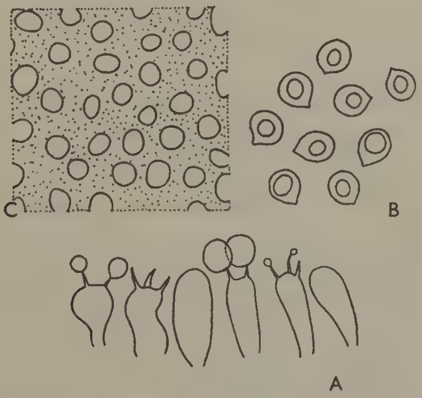

Fig. 3. *Fomes annosus* (Fr.) Cke var. *indicus*. A. Basidia, × 1150. B. Apiculate basidiospores with a small guttule, × 1150. C. Pores, × 20.

Habitat on pine stumps, Tanhaulti, Mussoorie, coll. K. S. Thind, 11 Sept. 1954. **227** (Fig. 3, A–C).

Other specimens preserved in the Kew Herbarium: on silver fir (*Abies pindrow*), Deoban, U.P., coll. R. S. Hole, June 1922; on spruce (*Picea morinda*), Jaunsar, U.P., coll. R. S. Hole, 7 Oct. 1922 [lectotype]; on spruce (*P. morinda*), Jaunsar, U.P., coll. R. S. Hole, 12 Oct. 1922; on cedar (*Cedrus deodora*), Kulu, Punjab, coll. Goran Ditta Mal; from roots of decomposed stump of spruce (*P. morinda*), Mundali, Chakrata Division, U.P., 8300 ft., coll. K. Bagchee, 3 Oct. 1935.

This variety is easily recognized by the small size of the pores. These are 4–5 per mm. in var. *indicus*, and 2–3·5 per mm. in var. *annosus* according to most European mycologists, although Overholts (1953) and Lowe (1955) give 2–4 per mm. for American material.

For an account of some diseases caused by *Fomes annosus* var. *indicus* see Butler (1903) and Hole (1927).

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STUDIES ON BASIDIOMYCETES IN SOIL

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The isolation of Basidiomycetes from soil has been studied. While extensive series of dilution and soil plates from wheat-field and pasture soil failed to record these fungi, they were isolated from roots, and from hyphae, rhizomorphs, and sclerotia picked out from soil. Over a 3-year period, no basidiomycete fructifications were found in the wheat-field although isolations from soil and roots showed that the field had an abundant and varied population of Basidiomycetes. While fructifications were obtained from the pasture, the species thus seen fruiting were different from those isolated from soil and roots, showing that the population was more varied than a study of fructifications alone would suggest. Some Basidiomycetes have been induced to fruit in culture.

Introduction

Basidiomycetes flourish in field and forest soils, and many surveys of the occurrence of their characteristic fruit-bodies have been made. In this way a large body of data on their taxonomy, habitat, geographical range and seasonal occurrence has been accumulated. Very little is known, however, about the occurrence and growth of their mycelia in soil. Mycelia bearing clamp connexions have been conspicuous by their absence in soil fungal studies, and while sterile mycelia suggestive of these fungi, but without clamps, have been recorded, the difficulty in obtaining fructifications in culture has precluded their positive identification as Basidiomycetes. Many workers have commented on this paucity of information on Basidiomycetes in soil, and Chesters (1949) has called them 'the missing link in soil mycology'.

During an investigation on the occurrence and activity of fungi in a wheat-field soil (Warcup, 1957), Basidiomycetes were recorded by the hyphal isolation method, whereas they were absent from soil and dilution plates prepared from the same soil samples. Microscopic examination of soil also showed basidiomycete rhizomorphs on the bases of wheat plants and on buried residues. Further data on the occurrence and isolation of Basidiomycetes are presented in this paper. The investigations were carried out on a wheat-field and on a pasture soil at the Waite Institute, Adelaide, South Australia; details of climate and soil have already been given (Warcup, 1957).

Criteria used to distinguish basidiomycete mycelia

Since Basidiomycetes are rarely known to fruit in agar culture, criteria besides that of fruit-body production are necessary to distinguish these fungi from the many other sterile cultures isolated from soil, especially by the hyphal isolation method (Warcup, 1955). In this work the following criteria have been used:

(1) Presence of clamp connexions.
(2) Presence of rhizomorphs (particularly under natural conditions).
(3) Duplication with a culture from a known Basidiomycete.
(4) Production of fructifications in culture.

Clamp connexions are usually considered to be typical of the Basidiomycetes, though Bessey (1950) states that early investigators reported clamps in *Tuber*. While the presence of clamps is the simplest and most useful criterion of a basidiomycete culture, it is well known that many Basidiomycetes do not form them, also that monocaryotic mycelia lack them. The presence of rhizomorphs has been a useful character for some Basidiomycetes, particularly in making visual or microscopical examination of soil or residues. With one exception, all the fungi isolated from rhizomorphs also produce clamp connexions. Nevertheless, this character is not of unequivocal value because some Ascomycetes produce rhizomorphs. While comparison of isolates with cultures from known Basidiomycetes, as suggested by Chesters (1949), would seem a good method, it has difficulties in practice. A wide range of fructifications must be obtained to make a reference collection of cultures, a task that may take several years. Besides, random isolates from fructifications have been found to differ in growth rate, and growth rate may change on continued cultivation, and this affects colony appearance and makes difficult a definite identification. Thus it is not certain that the basidiomycete groups separated in this study, although they represent different cultural types, are in fact all different species of fungi. The production of typical basidiomycete fructifications is the most satisfactory means of identification of cultures, and has been the aim throughout this study, though, as yet, only six species have been induced to fructify in culture.

While more than 170 different sterile fungi have been isolated from wheat-field and pasture soils, only thirty-two may be referred to the Basidiomycetes, using the above criteria. Several other isolates have some cultural characteristics which suggest that they too may be Basidiomycetes, but since more definite proof is lacking, they are not considered as such in this paper.

Isolation methods

Data to be given in this paper on the isolation of Basidiomycetes from soil and roots have come both from general investigations where the aim has been to study the fungal population as a whole, and from specific studies where Basidiomycetes alone have been considered. General methods have included dilution plates, soil plates, hyphal isolation, isolation from roots, and isolation of sclerotia from soil; specific methods have been the isolation of rhizomorphs from soil, residues or roots.

Dilution plates at a dilution of 1:5000, soil plates, and hyphal isolation plates were prepared as described by Warcup (1957). Sclerotia were obtained from soil either by sieving or by using nematological flotation methods, either the simple conical flask method, or the Fenwick can

(Goodey, 1957). Rhizomorphs were plated in two different ways, depending on soil moisture at the time of their collection. If the soil was very dry (below wilting point), rhizomorphs were picked from soil or debris with sterile forceps, shaken free of any attached soil particles, and plated on the surface of dried agar plates; if the soil was moist, rhizomorphs were washed in sterile water, and then plated in the same manner as small roots.

Isolations from roots were made using a root-fragmentation method. Roots were gently washed free from soil, were further washed in several changes of sterile water, and then examined under a binocular dissecting microscope (× 12·5 and × 25). For fungal isolation small pieces of root, about 3–15 mm. in length (the length depending on the diameter of the root sampled), were cut off and placed in a 7 cm. diam. isolation dish. Wide roots were usually dissected (Waid, 1956) into two parts, outer cortex, and stele with some inner cortex, each part being placed in a separate dish. Before pouring the plates with melted but cooled agar, each root piece was dissected into small fragments with two needles under the microscope; the fragments were then dispersed throughout the medium by shaking and rotating the plate before the agar solidified. They were then located and ringed on the reverse of the plate, and the plates were incubated at room temperature. By examining fragments under a microscope (× 120), it was possible in many cases to discern both the region of the root from which growth commenced and the nature of the fungal unit.

The medium used was Dox + yeast agar; for dilution and soil plates it was acidified to pH 4·2–4·4 with phosphoric acid; for hyphal isolation plates and for isolation from roots it was used unacidified and diluted to 1/6 normal strength. In the latter part of the work, streptomycin was used extensively with both dilute and normal medium to suppress bacterial growth on isolation plates. Streptomycin was added to the cooled but molten agar just before the plates were poured at 30 μg./ml. (Martin, 1950). Plating on to sterilized wheat straw also proved a useful method for freeing cultures from bacteria, since many Basidiomycetes grew well on straw whereas bacterial growth was slight.

ISOLATION OF BASIDIOMYCETES FROM SOIL AND ROOTS

A summary of isolations of Basidiomycetes from wheat-field and pasture soils is given in Table 1. While Basidiomycetes were obtained from roots, and from hyphae, rhizomorphs, and sclerotia isolated from soil, they were not recorded by either the dilution or the soil plate method. Many previous investigators (Brierley, 1923; Bisby, Timonin & James, 1935; Chesters, 1949) have also reported that Basidiomycetes are not recorded in dilution plate studies. The observation that basidiomycete mycelium (particularly rhizomorph fragments) may float, or sink enmeshed with the heavier soil particles, rather than stay in suspension, may partly explain why Basidiomycetes are seldom recorded on dilution plates.

Although Basidiomycetes were not recorded from soil plates in this study, Warcup (1951 *a*) isolated Basidiomycetes, mainly 'fairy-ring' fungi, from natural grassland soil by this method; Basidiomycetes have also been isolated from forest nursery soil at Ampthill (Warcup, 1951*b*), and from natural fen soil at Wicken (Stenton, 1953). A possible explanation of these differing results may be that where Basidiomycetes have been successfully isolated, the number of colonies of other fungi on the soil plates has been unusually low. With the fairy-ring fungi studied, it was found that few fungi were present on plates prepared from the mycelial zone of the Basidiomycete; at Ampthill, and at Wicken, Basidiomycetes were isolated predominantly from the lower levels of the profile where the number of colonies was low. While most Basidiomycetes so far investigated grow on Dox + yeast agar, often their slow initial growth makes them susceptible to competition on agar plates.

Table 1. *Numbers of Basidiomycetes isolated by various methods from wheat-field and pasture soils*

	Wheat-field			Pasture		
		No. Basidiomycetes			No. Basidiomycetes	
Method of isolation	No. plates examined	Colonies	Species	No. plates examined	Colonies	Species
Dilution plates	1059	0	0	441	0	0
Soil plates	690	0	0	294	0	0
Hyphal isolation plates	566	62	9	243	13	6
	No. units examined			No. units examined		
From sclerotia	48	25	2	6	2	1
From rhizomorphs	36	11	4	4	4	1
From wheat bases	783	105	8	—	—	—
From roots	—	—	—	319	139	12
Total no. species	—	—	19	—	—	16

Basidiomycetes were isolated by the hyphal isolation method; although the number of colonies obtained was not high, being about 1 % of the total isolated from hyphae, nine species were obtained from the wheat-field and six from the pasture. A few hyphae with clamps failed to grow on hyphal isolation plates; usually it was not possible to judge whether such hyphae were alive but incapable of growth on the medium, or whether they were dead.

Several Basidiomycetes were isolated from rhizomorphs picked from the soil mass, but most rhizomorphs were obtained from residues buried in soil or from the bases of wheat plants. Of 783 wheat plants collected at random from the field during the summer of 1955–56, 116 showed rhizomorphs at their bases and Basidiomycetes were isolated from 105 of these. Many of the rhizomorphs isolated from soil, particularly from moist soil, were found to have contaminating organisms on their surface, even after repeated washing.

Basidiomycetes were also frequently isolated from the pasture by the root-fragmentation method. Roots examined included those of pasture plants, mainly *Phalaris tuberosa* L., *Trifolium subterraneum* L., and *Bromus* spp., and also mycorrhizal rootlets of *Pinus* which occurred in the upper soil layers of the pasture near a row of *P. radiata* D. Don and *P. canariensis* C. Sm., at the edge of the field. Using the root-fragmentation method

Basidiomycetes, presumably mycorrhizal species, were isolated readily from the mycorrhizal roots. Basidiomycetes identical with those obtained from mycorrhizal roots were also isolated from adjacent dead and decomposing grass roots. The data obtained were not sufficient to tell whether these fungi were playing a part in decomposition.

Table 2. *Distribution of Basidiomycetes within the tissues of roots from pasture soil*

	No. roots containing Basidiomycetes	
Root zone	Live roots	Dead roots
Surface	1	4
Outer cortex	3	14
Stele	2	34
No. roots examined	126	173

The distribution of Basidiomycetes in roots from pasture soil is shown in Table 2. Most isolations were from dead roots, and a further feature was their frequent isolation from steles but relative absence from cortical tissue. This result does not seem to have been due to suppression of Basidiomycetes by faster-growing fungi on isolation plates, since the number of other colonies obtained was relatively low; moreover, hyphae bearing clamps were only occasionally seen during microscopical examination of fragments of cortical tissue. This result would also suggest that Basidiomycetes may have been even more common in the wheat bases and roots than surface examination showed. These results on the isolation of Basidiomycetes from roots contrast with those obtained by Waid (1957) in a study of the fungi occurring in decomposing ryegrass roots. Although Waid frequently saw clamp-bearing hyphae in microscopical studies of semi-decomposed cortical tissues, he obtained these fungi on only two occasions in platings on agar of segments of whole or dissected roots. Plating root segments favours any faster-growing fungi present, whereas when roots are fragmented there is a greater chance of uncontaminated growth of slower-growing species from isolated root fragments. Besides the difference in technique, Waid's comment that no fungi were observed either penetrating the endodermis or within the steles of roots possessing a cortex, suggests that the roots studied by him were less decayed, and possibly contained less basidiomycete mycelium than those sampled here.

These data show that Basidiomycetes may be isolated from soil by a variety of methods, though they are unlikely to be obtained by dilution or soil plate studies. The results also suggest that any investigation, on Basidiomycetes in soil, that neglects to sample roots and larger debris is unlikely to be satisfactory.

IDENTITY OF THE BASIDIOMYCETES ISOLATED

When it became evident in 1956 that several Basidiomycetes were present in the wheat-field soil, information on their identity was sought by attempting to obtain fructifications on agar or on sterilized soil-straw mixtures in 500 ml. flasks, and by collecting fructifications to obtain a collection of named cultures for comparison with isolates from soil. Both methods were relatively unsuccessful; only two fungi fruited in culture, *Coprinus micaceus* (isolated from a fructification), and *Lepiota* T 156; although sixteen cultures were obtained from fructifications collected from adjacent pastures (none were found in the wheat-field), not one proved similar to the isolates from soil. Further isolations from fructifications made in 1957 increased the number of cultures to thirty-one and enabled three isolates, *Boletus granulatus*, *Naucoria semiorbicularis* and *Cyathus olla*, to be identified.

Table 3. *Species of Basidiomycetes obtained from wheat-field and pasture soils*

Wheat-field	Pasture
Species fruiting in the field	
None	*Boletus granulatus* Fr. *Clitocybe flaccida* var. *lobata* Fr. *Coprinus plicatilis* Fr. *Galera crispa* Longyear *Lepiota cristata* (A. & S.) Fr. *Lycoperdon hiemale* Bull. *Naucoria semiorbicularis* Bull. ex Fr. *Psalliota campestris* Fr. *Psathyra sonderiana* Berk. *Psilocybe foenisecii* Pers. ex Fr. *Stropharia* ? *obturata* Fr. *Hygrophorus* sp. *Panaeolus* sp.
Species isolated from soil or roots	
Coprinus T 198 *Cyathus olla* Pers. *Naucoria semiorbicularis* *Pellicularia filamentosa* (Pat.) Rogers *Sphaerobolus stellatus* Tode ex Pers. (T 150, T 153) ? *Lepiota* T 156 Cultures T 14, T 36, T 46, T 80, T 116, T 144, T 146, T 147, T 149, T 151, T 154, T 155, T 172	*Boletus granulatus* Fr. *Marasmius* T 260 Cultures T 144, T 147, T 151, T 172, T 216, T 224, T 230, T 234, T 235, T 248, T 251, T 252, T 280, T 282, T 285

Attempts were then made to obtain fructifications under semi-natural rather than pure culture conditions. Cultures were grown on sterilized wheat chaff in large tubes until the fungus thoroughly permeated the medium, were then removed from the tube, placed in earthenware pots, and covered with sand or soil as a 'casing soil' and kept under humid conditions. This method has shown the most promise of any yet tried, and fruit-bodies of *Lepiota* T 156, *Cyathus olla*, *Sphaerobolus stellatus*, *Marasmius* T 260, and *Coprinus* T 198 have been obtained. Several types of 'casing soil' were tried, wheat-field soil, coarse and fine sandy soils, all sterilized and unsterilized; none was entirely successful, but, so far, the unsterilized fine sandy soil has given the best results. *Trichoderma* and *Penicillium* may invade and overrun cultures, particularly when the casing soil has been sterilized. On the other hand with unsterilized soil, Discomycetes and occasional Coprini have fruited as contaminants. In all cases where fructifications were obtained, the fungus was isolated from the fruit-body for comparison with the original culture used.

A list of all Basidiomycetes obtained from wheat-field and pasture, either from fructifications or by isolation, is given in Table 3. Identifications follow Cunningham (1944) for Gasteromycetes, and Cleland (1934) for all other groups.

ECOLOGY OF BASIDIOMYCETES IN WHEAT-FIELD AND PASTURE

Comparatively little is known about the occurrence of Basidiomycetes in agricultural soil, and general opinion seems that expressed by Russell (1950) who states 'Basidiomycetes certainly occur in some soils, particularly forest and grassland soils, though whether they are present in appreciable numbers in old arable soils is uncertain'. Glynne (1935) noted that white mycelium belonging to an agaric of the *Cortinarius* type was frequent on the roots and basal parts of both wheat and barley at Woburn.

Although no fructifications of Basidiomycetes were found during three years observation on the wheat-field studied here, isolation has shown that a high population of Basidiomycetes is present. Several Basidiomycetes from the wheat-field form sclerotia and one species (T 144) was found present as hyphae during the wet winter period and as sclerotia during the dry summer period. Rhizomorphs in soil or on plant remains may remain viable over the dry period. Rhizomorphs on the basal parts of wheat were first noted when the ears were starting to ripen, and, in 1955–56, by the time the crop had been harvested 14 % of the plants were showing visible rhizomorphs. Basidiomycetes have also been noted to colonize fresh wheat leaves if they are buried near rhizomorphs after ploughing. Most isolations of Basidiomycetes were from the surface 3 in. of wheat-field soil, but Basidiomycetes were often found present where residues had been deposited lower down.

The common Basidiomycetes recorded from the wheat-field were T 147, T 146, *Pellicularia filamentosa*, *Cyathus olla* and *Sphaerobolus stellatus*. The data on *P. filamentosa* are interesting and provide information on the ability of this fungus to live as a saprophyte. With one exception, a strain which was non-pathogenic to the hosts used (wheat, radish, lettuce and tomato), all isolates belonged to the crucifer strain (Flentje & Saksena, 1957) and did not attack wheat. Warcup (1957) obtained *P. filamentosa* by the hyphal isolation method from wheat-field soil, during the period of decomposition following ploughing. Further study has shown that it is a frequent and early invader of buried leaf tissue of wheat and grasses. Since young seedlings are abundant at ploughing, this ability to invade young tissue could account for its isolation after ploughing. Although non-pathogenic to wheat, the crucifer strain of *P. filamentosa* was found to be common on basal parts of young wheat plants. *P. filamentosa* was not recorded from pasture soil. The other common Basidiomycetes were found as rhizomorphs on debris, in soil, and particularly on mature wheat. The evidence suggests that such fungi are highly important in decomposition of straw and other resistant material added to soil.

In contrast to the absence of fructifications from the wheat-field, several fungi fruited abundantly in the pasture during the autumn-early winter period, after the beginning of the seasonal rains. The most abundant species recorded from fructifications were *Psalliota campestris*, *Lepiota crispa*, and *Naucoria semiorbicularis*; none of these species was isolated from roots or soil during these investigations. The Basidiomycetes isolated from pasture were found predominantly in roots, particularly dead and decomposing roots; they were also found occasionally on the surfaces, or in the cortex of living roots, and as hyphae or sclerotia in the soil mass. It was interesting that, in some *Phalaris* roots, decomposition of the vascular tissue had proceeded further than that of the cortex. In extreme cases a cylinder of outer cortex enclosed a thin strand composed of fibre cells, other cell debris, hyphae of Basidiomycetes, and often hyphae of phycomycetous endophytes.

The data for both wheat-field and pasture show that more Basidiomycetes may be present in soil than commonly fruit. Lange (1948), after a 3-year study of the fructifications of agarics of Maglemose, considered that that period was not sufficient to record all the species in the region, and Parker-Rhodes (1955) has estimated that he has obtained about half the estimated number of species of Basidiomycetes present on Skokholm Island after 5 years' study.

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A MODIFIED LA RUE CUTTER FOR SELECTING SINGLE SPORES AND HYPHAL TIPS

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(With Plate 2)

In a method of isolating single spores described by C. D. La Rue (*Bot. Gaz.* **20**, 319–320, 1920), a device resembling a microscope objective, but fitted with a circular cutting tip, was screwed into the revolving nosepiece of a microscope. Spores were spread on the surface of agar in a Petri dish placed on the microscope stage, and well separated single spores were located through a suitable objective. The cutter was then swung round in place of the objective and, when racked down on to the dish, it cut a disk of agar bearing the required spore.

The technique provides a ready means not only of isolating single spores, but also of obtaining hyphal tips. To obviate difficulties, however, in sterilizing the tip of the cutter and replacing it if it becomes damaged, a modified apparatus has been designed. It is described below and illustrated in Pl. 2.

The body (B) of the apparatus (A), which screws into the revolving nosepiece of the microscope, is hollow and made of duralumin. A brass cylinder (C) bearing the cutting tip (D) slips into this body and is held in place by means of a spring-loaded ball engaging in a groove on the cylinder. The cutting tips are made of stainless steel and are fastened by means of a screw cap (E) into a recess in the end of the cylinder. They are thus easily replaced and can be made in various sizes. All parts are accurately machined to ensure good centring.

To sterilize the tip, the cylinder is slid from the body and the tip passed through a bunsen flame. Alternatively, it may be dipped in alcohol and the alcohol burned off, although care must then be taken that no unburned spirit remains inside the tube. The cylinder is then replaced in the body.

It is desirable that the disk cut in the agar shall be of slightly smaller diameter than the field of view of a $\frac{2}{3}$ in. objective. The cutting tips shown in the illustration have an internal diameter of 1·5 mm. and are bevelled externally to cut cleanly through hyphae. Both for isolating single spores and for obtaining hyphal tips, 3 % filtered plain agar is poured to a depth of about 3 mm. in the plates. The spores are spread with a bent glass rod and the surface of the agar allowed to dry before the spores are selected.

If hyphal tips are required, very small inocula (e.g. 1 mm.^{3}) are placed at several points on the agar. They are examined frequently during the next 12–48 hr. to detect the sparse hyphae that grow from them initially. It is then usually possible to find single hyphae which are well grown and branched and will transplant readily. Considerable care must be taken when selecting such hyphae to ensure that they are, in fact, single and that there are no others beneath them which would also be contained within the disk. This remains in the plate and is removed by inserting a sterile needle into the outer half (i.e. away from the centre of the colony) to avoid trapping other hyphae. The disk can be re-examined under the microscope before being placed in the subculture tube.

EXPLANATION OF PLATE 2

Modified La Rue cutter (A) and component parts (B–E). For explanation see text.

Note. The cutter as shown can be obtained from Mr I. Saint, 50 Clarendon Avenue, Leamington Spa, Warwick.

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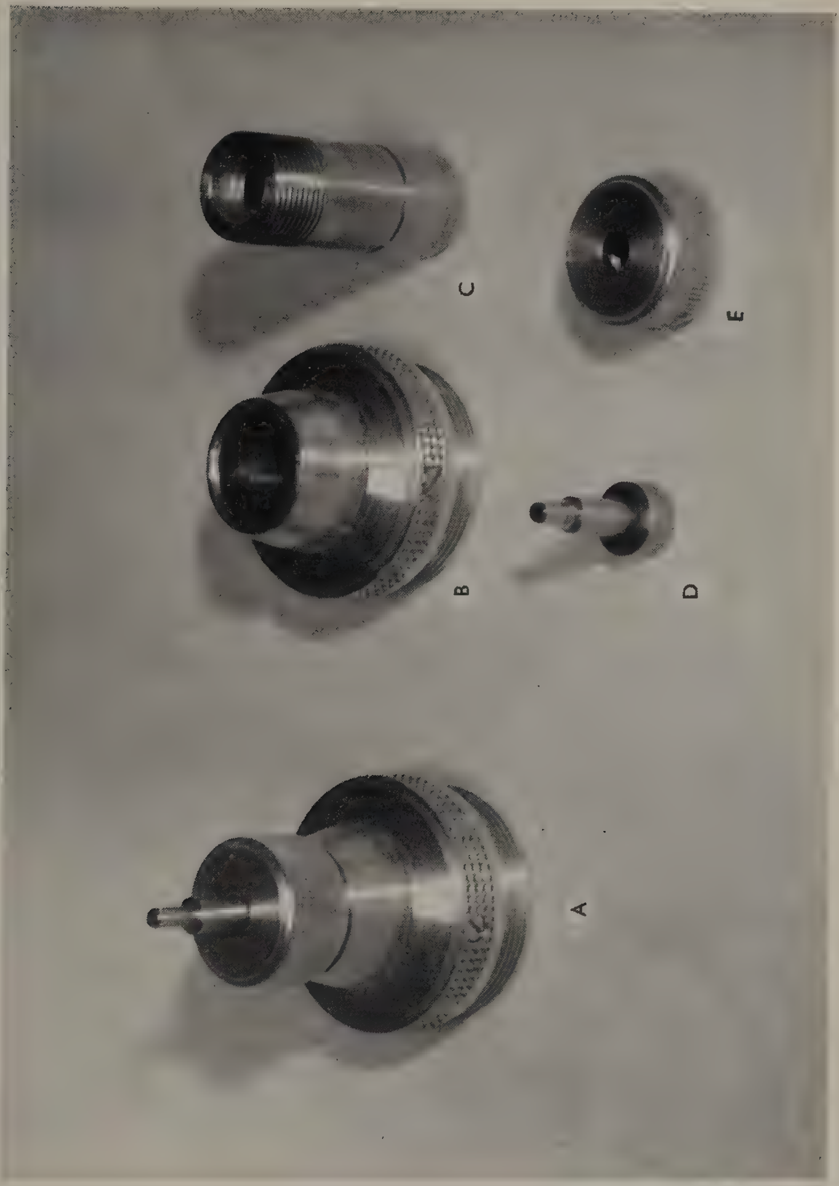
A
B
C
D
E

Trans. Brit. mycol. Soc. **42** (1), 55–58 (1959).

STUDIES ON *PUCCINIA POLYSORA* UNDERW.

III. DESCRIPTION AND LIFE CYCLE OF *P. POLYSORA* IN WEST AFRICA

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The form of *P. polysora*, the southern American maize rust, present in West Africa, is described. The apparent absence of aecidial and pycnidial stages and the failure of teleutospores to germinate when subjected to various chemical and physical stimuli suggest that the rust may be classified as a micro-cyclic, autoecious hemi-form.

DESCRIPTION AND HOST RANGE

PUCCINIA POLYSORA UNDERW. (*Bull. Torrey bot. Cl.* **24**, 86, 1897.)

Dicaeoma polysorum (Underw.) Arthur. *Result. Sci. Congr. int. Bot. Vienne*, 1905, p. 344, 1906.

Uredosori. Pustules small and circular, sometimes slightly elongated, becoming densely and uniformly distributed, cinnamon to yellowish brown, commonly opening by a longitudinal slit. *Uredospores.* Yellowish or golden, ellipsoid or oval, non-pedicellate, 27–41 × 20–29 μ, finely and sparsely echinulate, pores usually 5, sometimes 4, equatorial. *Teleutosori.* Subepidermal indehiscent, 0·5–1·5 mm. long, chocolate brown. *Teleutospores.* Usually angular from pressure, pedicels persistent, brown, 29–41 × 19–27 μ, wall 1·5 μ usually not much thickened above, pedicel short, mesospores common. *Aecidia* and *Pycnidia* unknown.

HOSTS. *Tripsacum laxum* Nash, *T. latifolium* Hitchc., *T. lanceolatum* Rupr., *T. dactyloides* L. (*T. monostachyum* Willd.), *Erianthus divaricatus* (L.) Hitchc., *Euchlaena mexicana* Schrad., *Zea mays* L.

SYMPTOMS OF *PUCCINIA POLYSORA* ON *ZEA MAYS*

The uredo- and teleutosori are found predominantly on the upper and, to a lesser extent, on the lower surface of the leaf, the husks of the ear and, rarely, on the most susceptible cultivars, on the true stem at the nodes.

Uredosori occur on both sides of the leaf. Each pustule is formed from an individual infection and the mycelium is restricted to the host tissue immediately beneath the pustule. The uredosori are scattered at random over the entire intervascular areas of the leaf. In severe infections the pustule density may be as great as 50 per cm.^{2} and the entire host plant may assume a bright orange appearance. The uredosori are generally circular, sometimes oval, 0·2–1·3 mm. diameter and never confluent.

When the uredosori are few and widely dispersed it is frequently seen that 9–10 days after the appearance of a pustule it becomes surrounded by a circle of secondary sori. A similar observation has been made with *P. graminis tritici* by Allen (1926), Arthur (1929) and Dodov (1931). Histological examinations of these infections show no spread of the original mycelium and apparently the secondary sori are initiated by infections from the primary sorus.

In susceptible cultivars the appearance of a green island, caused by the retention of chlorophyll in the senescent host tissue, is evident around the pustules and is retained for a considerable period after the death of the surrounding leaf tissue. A similar symptom has been described by D'Oliveira (1939, 1940).

The teleutosori are extremely rare in West Africa. The sori are generally slightly elongated, 0·5–1·5 mm. long, and chocolate brown in colour. They do not dehisce, remaining covered by the epidermis, and are difficult to detect.

LIFE CYCLE

Observations on the development of the rust were made on seedlings of *Zea mays*, inoculated with uredospores when 3 weeks old and grown in a glasshouse with a diurnal range of temperature of 70–95° F. (mean 85° F.) and saturated humidity.

Six days after the inoculation of a leaf surface with uredospores, faint, greenish yellow spots, approximately 1 mm. diameter, appear at the infection site. The spots gradually yellow towards the centre and begin to distend on the seventh day after inoculation. On the eighth or ninth day the epidermis ruptures, generally by a longitudinal slit, revealing the mature uredospores which are immediately infective.

The teleuto-stage has never been observed in the greenhouse and is exceptionally rare on adult plants in the field at Ibadan, teleutosori having been found only on a few mature plants at the senescent stage. In the rain-forest areas of Nigeria uredo- and teleutospores have never been found in the same sorus, but examination of material from the montane areas of Cameroons has shown transitional sori on maize growing at altitudes above 4000 ft.

The aecidial and pycnidial stages of *P. polysora* are unknown. During the course of observations on the rust a constant watch has been kept on associates of *Z. mays* in the field, but no related aecidia or pycnidia have been found, and in the American continent, the centre of origin of the rust, these stages are also unknown.

Experiments in attempt to induce germination of teleutospores

On the discovery of teleutospores in Nigeria in 1953, preliminary experiments were carried out in an attempt to induce germination and determine the importance of the teleuto-stage in the life cycle. All experiments were unsuccessful, as were similar tests in East Africa. A further series of experiments was designed at Ibadan and teleutospores were subjected to various chemical and physical stimuli 3, 6 and 12 months after collection. Since all tests gave negative results they are only summarized.

(*a*) Maneval (1922) induced germination of teleutospores by alternately wetting and drying host tissue bearing sori and then floating it on water for long periods. Material of *P. polysora* was wetted and dried for 2-day periods 5–20 times after storage in the refrigerator (10° C.), in sealed tins in the field, or in the laboratory (27° C.).

(*b*) Johnson (1931) attempted to break the dormancy of teleutospores of *P. graminis tritici* by freezing for 2–10 days, spraying with water at 5° C. for a week and then wetting and drying alternately for various times. Cooling for 5–20 days at 5° C. was tried with *P. polysora*, followed by wetting and drying for different periods and finally floating on distilled water.

(*c*) The method of Thiel & Weiss (1920) was tried both on fresh material and on material stored at 10° C. for various periods. Teleutospores were exposed to chloroform vapour in a closed jar for 1 min. and then immersed in 1% citric acid for 15 min.

(*d*) Fresh and previously frozen material were immersed in acetic acid at 0·1, 0·01 and 0·001 N for 15 min. to 5 hr.

(*e*) Sulphuric acid at the same dilutions was tried and material immersed for 5 min. to 24 hr.

(*f*) Several lipoid solvents, acetone, ether, chloroform and carbon disulphide were used in an attempt to increase the permeability of the spore wall to water and oxygen. Material was immersed in the undiluted solvents for 5 to 30 min. then washed in running water and floated on distilled water for 10 days.

The method of perpetuation of Puccinia polysora *in Nigeria*

For the rust to survive from one maize crop to the next it must endure two inter-crop periods each year. The general practice in the south of Nigeria is to plant the main crop in early April and harvest in June, and the second crop in early September and harvest it in November. The continuity of infection from the first to second season crops presents no difficulty owing to the wide latitude of planting dates encountered in local husbandry methods, and direct infection of the second crop could occur from mature, late planted first season crops.

The infection of the early crop each year necessitates the rust surviving the dry months from November to April, during which period maize is not normally grown, and this period is far in excess of the maximum viability of the uredospores as determined experimentally. A search was made for alternative hosts and *Tripsacum laxum*, maintained in small experimental plots at various agricultural stations throughout Nigeria, was examined during the dry months but no infection found. Collections of rusts on grasses were made during the dry seasons of 1953–55 and none proved to be *P. polysora*. It was determined, however, with an automatic volumetric spore trap (Cammack, 1958) that viable uredospores of *P. polysora* were present in the atmosphere throughout the dry months, indicating that a source of inoculum capable of infecting the first-season maize crop was present in the atmosphere when the crop reached a susceptible stage of growth.

During these observations the writer observed that in the south-west area of Nigeria and in the freshwater swamp communities on the coast it was a local procedure to grow 'out of season' maize in small irrigated plots on the banks of streams, and these were often seen to be heavily rusted. The first reports of rust on first-season maize came from this area each year and it is most likely that rust is carried through the dry seasons in this way, infects the local first-season maize crops, which are generally planted early in March in the south, and eventually spreads northwards each year, the inoculum being carried on the prevailing south-west monsoon wind.

This method would provide a simple system of perpetuation of the rust during the dry months and would also explain the presence of low levels of viable uredospores in the atmosphere during that period.

Discussion

It is likely that *Puccinia polysora* is a microcyclic rust, the aecidial and pycnidial stages being absent. This must be only a hypothesis at present since extensive inoculation studies have not been done, either in the presumed area of origin of the rust, the Americas, or in any of the territories now affected by the rust. The view that the rust is a microform is strengthened by the failure to germinate teleutospores under experimental conditions which were, however, far from comprehensive. It is possible that, in the course of evolution of the host-parasite relationship, the pathogen has become separated from its aecidial host and that the teleutospore has in consequence lost the capacity to germinate. On examination of the nucleus after each test it was observed to have remained in the expanded 'resting' phase (Savile, 1939) and unchanged in appearance from the untreated spore.

The rust is highly successful in the uredo- stage and the ease of continuity of this stage in a tropical environment may have resulted in the suppression of the sexual phase in the life cycle. In the absence of further evidence *P. polysora* may be classified provisionally as a micro-cyclic, autoecious hemi-form.

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OOSPORA PUSTULANS OWEN & WAKEFIELD AS A PARASITE OF POTATO ROOT SYSTEMS

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(With Plates 3 and 4)

A fungus isolated from brown lesions in the cortex of root systems of Majestic potatoes at Rothamsted in 1951 was identical morphologically with *Oospora pustulans*, the cause of skin-spot disease of tubers. The pathogenicity of *O. pustulans* from tubers for root systems of potato and tomato was demonstrated. Tuber yield was not greatly affected despite severe damage to the roots, and the chief importance of root infection may be to provide sources of infection for progeny tubers or subsequent potato crops.

The infection of root systems, prevalent at Rothamsted each year, does not obviously depend on previous potato cropping, or on the presence of macroscopic skin-spot symptoms on the seed tubers. Observations suggest that eye infections, which may not be detected by macroscopic examination, are common and that soil-borne fungus is probably less important in leading to outbreaks of skin-spot than fungus on the seed tubers. Tests with tomato seedlings should help to determine how long *O. pustulans* survives in soil.

The 'skin-spot' disease of potato tubers was named by Pethybridge (1915) in Ireland, but was probably described earlier on tubers from Lincolnshire (Carruthers, 1904). From the small pimple-like spots that developed on the surface of the stored tubers, Owen (1919) consistently isolated an unidentified fungus which was subsequently named *Oospora pustulans* Owen & Wakefield. The first attempts to infect tubers artificially were rarely successful, only a few spots developing in several experiments (Owen, 1919), but Millard & Burr (1923) obtained skin-spots abundantly on stored tubers that had been grown in sterilized soil inoculated soon after the plants emerged.

The ability of *O. pustulans* to attack and kill 'eyes', first recognized by Milburn & Bessey (1915), or its presence on stolon scars (Chamberlain, 1935), were not considered important for its perennation because the fungus was assumed to be a soil inhabitant, which infected tubers when they became mature (Greeves & Muskett, 1939; Allen, 1957; Boyd, 1957). This view was supported by the fact that the disease appeared year after year on tubers from certain soils, farms or even fields. However, there were no records of the fungus being isolated from soils, or information as to how long it would persist there under various conditions.

In 1951 the roots of Majestic potatoes growing at Rothamsted were found to be brown; a fungus was isolated, from the discoloured cortical tissues, which Dr F. J. Moore and Dr M. B. Ellis considered to be morphologically indistinguishable in culture from *O. pustulans*. When similar symptoms were also found commonly on potato plants in other parts of the country, it was decided to test whether the fungus isolated from roots had the same pathogenicity as *O. pustulans* from skin-spots on tubers, because it seemed that root infections might be important in the life cycle of the fungus and affect potato yields.

SYMPTOMS

Brown lesions were found on all parts of the root system of potato plants, and we have assessed their extent on the underground part of the stem ('stem base') and on the stolons and adventitious roots ('roots') which develop from it. The lesions, which are often well developed when the shoots emerge above ground, are at first small, discrete, light-brown spots. They coalesce forming large brown patches, which sometimes occupy the whole cortex but do not penetrate deeper (Pl. 3, fig. 1). Later the colour darkens and small transverse cracks appear in the epidermis; eventually deep and wide longitudinal cracks may develop in the cortex, which can then be detached easily to expose the clean vascular cylinder (Pl. 3, fig. 2). Symptoms often appear first and are most severe where the stem base joins the seed tuber, or on the stolons and roots arising from this region. Lesions are rare on growing tubers, and no above-ground symptoms are known.

Transverse sections through infected stem bases (Pl. 4, fig. 4) show fine branched hyphae which penetrate cell walls in the cortex but do not extend into the phloem. Some lesions are restricted by the formation of irregular patches of wound cork cells produced by septation within the lumen of cortical parenchyma cells (Pl. 4, fig. 2). Where deep longitudinal cracks penetrate the cortex, similar meristematic activity occurs within the outer layers of the phloem (Pl. 4, fig. 3).

ISOLATION AND GROWTH IN CULTURE

Fructifications, identical with those of *O. pustulans* from infected tubers, are produced abundantly within 2–3 days when infected root systems are kept damp at 15–20° C. (Pl. 3, figs. 3, 4), and we have used 'sporing tests' extensively in confirming the presence of the fungus in suspected lesions. The fungus was isolated in pure culture both from conidiophores, and from infected cortex soaked for 1 min. in 1 % silver nitrate, washed in sodium chloride, then in water, and plated on potato-dextrose agar. Surface sterilization with 0·1 % mercuric chloride reduced the number of contaminants but also usually prevented the isolation of *O. pustulans*.

Measurements of growth at different temperatures, determined from the dry weight of a root isolate after 14 days growth on 20 ml. potato-dextrose broth in 250 ml. conical flasks, agreed closely with observations by Owen (1919) and Fuchs (1954) for isolates from tuber pustules. Colonies of two root- and two tuber-isolates on potato-dextrose agar grew radially at similar rates; all grew fastest between 16° and 18° C., above 20° C. growth was much less and it ceased at 25° C.

PATHOGENICITY

Isolates of the fungus from root systems, and of *O. pustulans* from skin-spots on tubers could reasonably be considered the same if both caused similar symptoms on roots and tubers. There are two difficulties in proving this point experimentally. First, detailed examination showed that many tubers although free from skin-spots had minute infections with *O. pustulans* on the bud-scales of the rudimentary sprouts (Allen, 1957), so it was impossible to rely on control tubers being free from the fungus. Secondly, experimental infection of clean tubers free from wounds requires careful control of storage conditions, although it occurs readily in normal clamp storage.

Experimental methods

For inoculation, the fungus was applied to tubers or soil as suspensions of a macerated sporing culture grown for 2–3 weeks on 25 ml. potato-dextrose broth. For inoculating soil the suspensions were diluted with 25 times their volume of water; 100 ml. was applied to the surface of each pot and lightly watered-in. Seed tubers were inoculated by suspensions mixed with soil to give a slurry of creamy consistency which adhered to the tuber during planting.

After a period of growth long enough for lesions to appear, or after complete growth of the crop, plants were removed from the soil and the root systems washed and examined. The extent of fungus attack on various parts of the root system was estimated, treating each stem base, its roots and stolons as separate units, although several such groups may have arisen from a single tuber to produce the potato hill. Each unit was awarded marks according to the following scale:

Marks	Infection category	Extent of lesions	
0	Clean	No browning	
1	Slight	Less than $\frac{1}{3}$ browned	(Pl. 1, fig. 1)
2	Moderate	$\frac{1}{3}$ to $\frac{2}{3}$ browned	
3	Severe	More than $\frac{2}{3}$ browned	

From the total score a percentage disease rating (*DR*) for stem bases, stolons and roots was calculated:

$$DR = \frac{\text{Total marks}}{\text{No. stem bases} \times 3} \times 100.$$

Diagnosis was generally confirmed by a sporing test as described above. Notes were also made of the occurrence of other root pathogens; during active growth the most common was *Rhizoctonia solani*. Lesions caused by this fungus are readily distinguished from possible *Oospora* lesions by their deeper penetration and more discrete and dark coloured margin. *Colletotrichum atramentarium*, which was rarely found when plants were growing actively, sometimes became the dominant fungus on senescent plants, particularly on the roots, but our examination was usually made before this stage was reached.

Infection of potato root systems with isolates from tubers

The first test of pathogenicity was made in pots in a heated glasshouse between 17 May and 12 Aug. 1954. Plugs of tuber tissue, each bearing an eye, were punched with a cork borer from Majestic or King Edward tubers free from skin spots. Three of each were planted in a 6 in. (No. 32) pot in unsterilized soil (John Innes no. 1 potting compost). To expose more stem to infection each pot was fitted with a celluloid collar and an extra 2 in. soil added. Two isolates were used, 'R' obtained from a root of Majestic grown at Rothamsted in 1951 (I.M.I. 47720) and 'T' obtained in 1954 from a skin-spot on a King Edward tuber. The following five treatments, each replicated four times, compared plants grown in soil without inoculation and in soil inoculated (with either 'R' or 'T' cultures) at planting and at three fortnightly intervals thereafter. The plants were examined 12 weeks after planting. The uninoculated controls (Table 1) showed some lesions, and a sporing test confirmed the presence of *O. pustulans* on about half of these on stems and stolons and on one-tenth of these on roots. It is impossible to say whether these plants were infected from fungus in the soil, from chance contamination, or from inconspicuous lesions on the seed pieces, for although no tubers with skin-spots were used, the bud-scales of the eyes were not closely examined. Both isolates increased the disease rating equally on both cultivars. Late inoculation had little effect compared with that soon after planting, perhaps because the fungus was given too little time to develop, because the temperatures were too high in the glasshouse in the later part of the experiment, or because there was a real decrease of suceptibility with age.

Table 1. *Pathogenicity of root and tuber isolates to root systems*

Time inoculated, weeks after planting	Isolate (R=root, T=tuber)	Disease ratings after 12 weeks: Means for all underground parts		Disease ratings after 12 weeks: Means for both cultivars			Percentage brown lesions on which *O. pustulans* spored		
		Majestic	King Edward	Stem	Stolon	Root	Stem	Stolon	Root
0	R	91	97	87	100	98	100	100	100
	T	95	87	86	90	96	100	100	100
2	R	82	86	86	80	87	100	100	96
	T	85	89	88	86	85	100	100	100
4	R	67	73	46	78	89	100	100	94
	T	56	40	17	46	64	75	94	82
6	R	32	35	21	35	46	92	100	100
	T	27	39	18	40	58	100	100	82
Uninoculated		22	21	14	16	34	60	60	8

A second comparison of the pathogenicity of root and tuber isolates to the root systems of five maincrop cultivars was made with whole tubers planted out-of-doors on 15 June 1954 in large pots, each holding 28 lb. unsterilized soil (John Innes no. 1). A single tuber, free from skin-spots, was planted in each pot, and four replicate tubers of each cultivar were allotted to each of the following treatments: no inoculum, isolate 'R', and a mixture of isolates from skin-spots on tubers of King Edward, Red King, and Majestic. The disease ratings, estimated after 12 weeks (Table 2), were similarly affected by inoculating with either the root or the tuber isolates. Ulster Supreme was less infected than the other cultivars, but again we cannot distinguish between the effects of possible resistance to infection and the extent of undetected eye infection on the five cultivars.

Table 2. *Pathogenicity of root and tuber isolates to root systems of potato cultivars inoculated at planting*

	Disease rating after 12 weeks								
	Control			Tuber isolate (T)			Root isolate (R)		
	Stem	Stolon	Root	Stem	Stolon	Root	Stem	Stolon	Root
King Edward	17	50	33	75	67	58	84	92	58
Red King	0	33	42	84	100	67	84	92	50
Majestic	0	17	8	89	89	56	84	84	66
Kerr's Pink	0	8	25	58	84	58	75	84	58
Ulster Supreme	0	0	0	50	75	58	42	67	58
Mean	3·4	21·6	21·6	71·2	83·0	59·4	73·8	83·8	58·0
	15·5			71·2			71·9		

In an attempt to produce plants free from *O. pustulans*, we grew true potato seedlings and found them susceptible to infection but difficult to handle and score. The latter difficulties were largely overcome by the discovery that tomato seedlings, which are larger and stronger, are also susceptible.

Infection of tomato roots with an isolate from potato tubers

The pathogenicity of a tuber isolate to roots of tomato seedlings was tested in an experiment also intended to show whether *O. pustulans* in field soils could be detected by growing tomato seedlings in them. In January 1955, soil samples were taken from two adjacent fields at Rothamsted which had been under permanent grass until a few years previously. In Great Field II the grass had been followed only by cereals, whereas in Great Field I the first potato crop, in 1954, had its roots severely infected.

To improve the texture of the soils for growth of seedlings, one part of sterilized sand was mixed with three parts of soil and samples from each field were inoculated with a tuber isolate as in earlier experiments. Four shallow earthenware pans (6½ in. diam.) were filled with inoculated and four with uninoculated soil from both fields, and sixteen tomato seeds (cv. Moneymaker) were planted in each. After 6 weeks in a heated glass-house the seedlings were washed and their tap roots examined. There were only a few small brown lesions on plants from uninoculated soils, whereas many seedlings from inoculated soils had completely brown and brittle tap roots, and swollen hypocotyls (Pl. 3, fig. 5). Several seedlings in inoculated soils showed 'damping-off' symptoms and survivors seemed to be retarded in growth. In a sporing test the fungus grew profusely from most inoculated seedlings, from some of those grown in the uninoculated soil from Great Field I which had grown potatoes in the previous year, but not from any seedlings from uninoculated soil in which potatoes had not been grown previously (Table 3).

Table 3. *Infection of tomato seedling roots by* Oospora pustulans *in uninoculated and inoculated field soils*

	After potatoes (Great Field I)		After cereals (Great Field II)	
	Control	Inoculated	Control	Inoculated
Percentage seedlings with browned tap-roots	5·6	71·0	2·0	87·4
Percentage browned roots from which *O. pustulans* was recovered by sporulation	67	91	0	98

Infection of tubers with a root isolate

Root isolates will grow on cut surfaces of tubers, and tubers can be infected easily through wounds. A field experiment in 1957 showed that root isolates could also increase the occurrence of skin-spots on the progeny of tubers inoculated before planting. Majestic seed tubers thought likely to be free from infection were selected in Northern Ireland in 1956, and the fungus was not found either on the skin or on the eyes of the tubers when these were examined carefully at Rothamsted. Twelve rows, each of thirty-six tubers, were planted in Great Field II on 29 April 1957. Tubers for half the rows were dipped in a slurry inoculum immediately before planting. By June, symptoms were well developed on the inoculated plants (disease rating 76) whereas uninoculated plants were clean (disease rating 0); by the end of September the disease ratings had risen in inoculated to 98 and in uninoculated to 27. In spite of the heavy and early infection of the inoculated plants, no difference between inoculated and control series was detectable in emergence, growth, or dying down of haulms. There were, however, fewer tubers in the inoculated rows and individual tubers were larger.

Samples of tubers were washed immediately after lifting, allowed to dry and placed in nylon nets in a potato clamp until 5 March 1958, when random subsamples of fifty tubers from inoculated and uninoculated rows were examined for skin-spots. Where the original seed tubers had been inoculated with a root isolate, many tubers bore large areas of skin-spots. The percentages of tubers falling into the categories none, trace, slight, moderate or severe, as defined by Boyd (1957), were respectively 32, 6, 24, 18 and 20 from inoculated, and 88, 6, 6, 0 and 0 from uninoculated rows.

Discussion

The results of the tests described above leave little doubt that *O. pustulans* is a parasite of potato root systems. The damage caused to cortical tissues is extensive and appears serious enough to reduce yield. Our experiments did not suggest any effect on tuber weight but they were not designed for this purpose and could have revealed only gross differences. If subsequent experiments confirm that yield is unaffected, the chief importance of root systems will appear to be in providing inoculum for infection of tubers or of subsequent potato crops.

Surveys of root systems of main crop potatoes at Rothamsted between 1953 and 1957 (Table 4) show that infection is prevalent each year and is not obviously dependent on previous potato cropping or on the presence of macroscopic skin-spot symptoms on the seed tubers. There were heavily infected crops on Great Field I in 1954 and on Highfield IV in 1957, although neither field had grown potatoes for very many years. Within the same field, different levels of infection have occurred on different stocks of the same cultivar, e.g. Majestic, Highfield 1957; or on different cultivars within a replicated experiment, e.g. Long Hoos 7, 1957. These observations suggest that soil-borne fungus is less important in practice than that introduced with the seed tubers. Development of the tomato seedling test should help to assess the amount of *O. pustulans* present during growth of potato crops and how long it is able to persist in the soil afterwards.

Table 4. *Incidence of* Oospora pustulans *on potato crops at Rothamsted, 1953–57*

Year	Field	Cultivar	Percentage stems showing			No. years since previous potato crop
			Clean	Moderate infection	Severe infection	
1953	Deacons	Majestic	38	20	42	5
	Garden Plots	Majestic	10	28	62	2
	Garden Plots	Gladstone	38	27	35	2
1954	Great Field I	Majestic	43	29	27	>80
1955	Garden Plots	Mean of 7	30	20	50	3
1956	Great Knott III	Majestic	21	15	64	7
1957	Highfield 4	Majestic (*a*)	18	22	60	>85
		Majestic (*b*)	59	14	27	
	Long Hoos 7	Majestic	75	2	23	>15
		Arran Viking	57	27	16	
		King Edward	95	4	1	
		Up to Date	98	0	2	

The infection of uninoculated plants grown from seed tubers devoid of skin-spots, and the prevalence of *Oospora* lesions at the point of attachment of stem bases to the parent tuber, suggest that eye infections are important. Severe eye infections may kill sprouts and so cause uneven emergence, whereas minute infections of bud-scales may infect the sprout without impairing its vigour, and so produce a higher percentage of infected root systems.

Widespread infection of the root systems of most plants in a crop during growth must provide ample inoculum for the infection of progeny tubers. Washing root systems before examination removes any fructifications and we do not know at what stage spores are first formed in soil, but we have found them occasionally on growing plants and profusely on senescent root systems. If it is true that spores are produced mainly on senescent plants, the inoculum would become most abundant as tubers reach maturity, when they are most suceptible to infection (Allen, 1957).

The lack of macroscopic symptoms on tubers at lifting is a difficulty in the detection and control of skin-spot disease. Assessment of the attack on the root system of crops grown for seed, or improved methods of direct examination for eye infections on seed tubers, may reveal stocks likely to become severely diseased. They may then be rejected as seed, or treated chemically at lifting and stored under conditions that prevent the development of severe symptoms on the eyes and skin surface.

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EXPLANATION OF PLATES 3 AND 4

Plate 3

Fig. 1. Stem bases of Majestic, showing cortical lesions due to natural infection by *Oospora pustulans*. (*a*) Slight; (*b*) moderate; (*c*) severe.

Fig. 2. Severely infected stem base, showing cracked cortex.

Fig. 3. *O. pustulans* sporing on an infected root after incubation.

Fig. 4. *O. pustulans* sporing on infected stem base, showing branching habit.

Fig. 5. Tap-root lesions on tomato seedlings grown in soil inoculated with *O. pustulans*.

Plate 4

Fig. 1. Transverse section of uninfected part of potato stem base, showing normal cortical parenchyma.

Fig. 2. Transverse section through lesion 1 in. below on the same stem, showing septation within parenchyma cells to form cork layer below superficial infection.

Fig. 3. Cork formation in the outer layers of the phloem, where the cortex has developed a deep longitudinal crack.

Fig. 4. Transverse section of infected stem base, showing mycelium in cortical cells.

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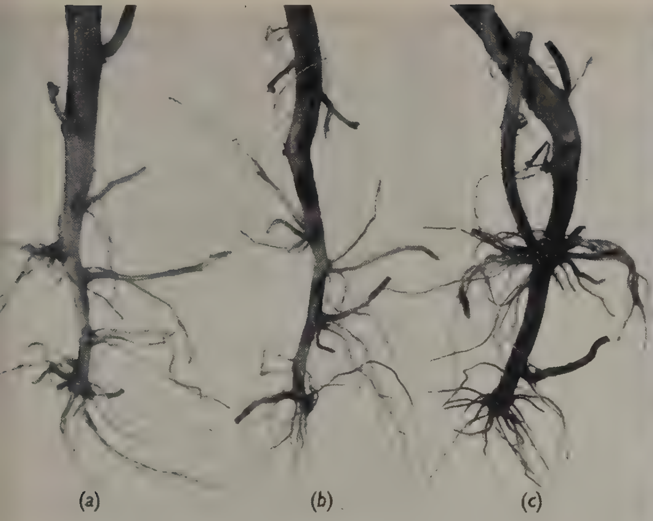

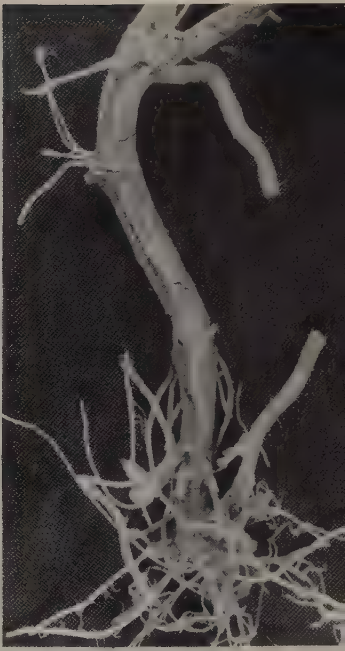

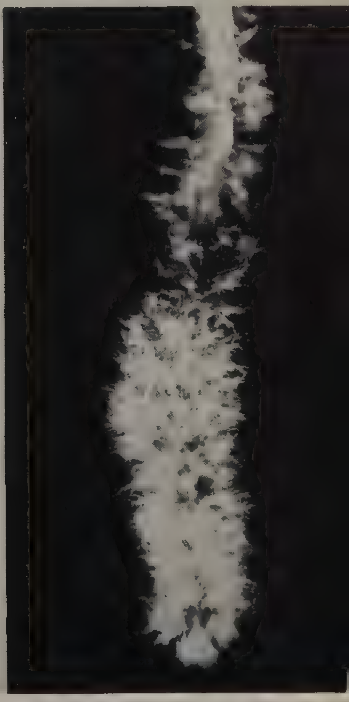

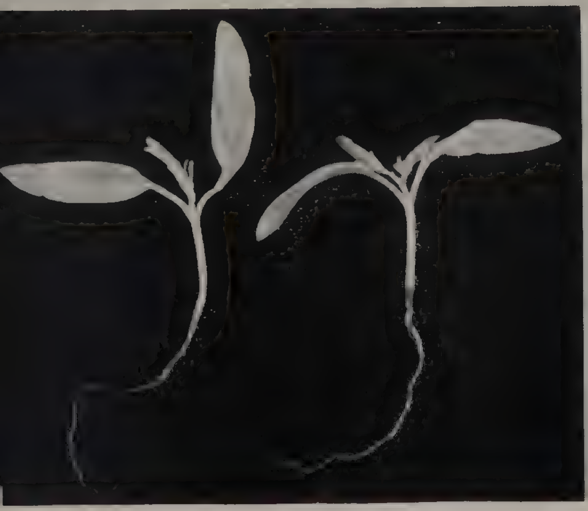

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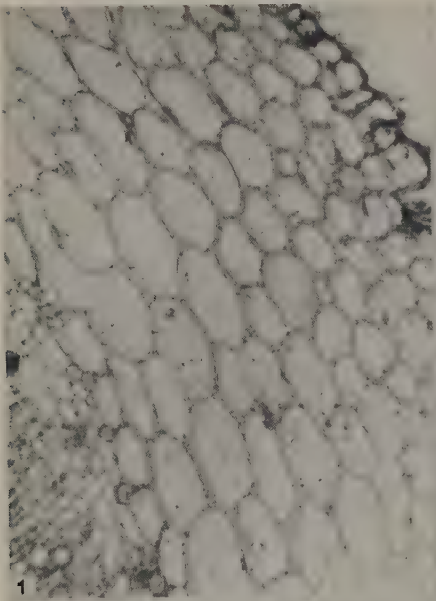
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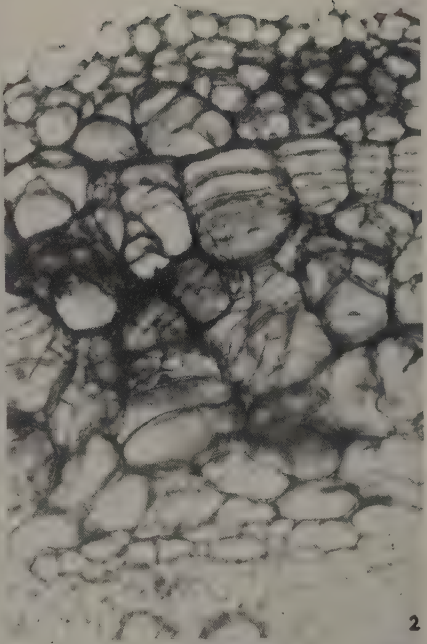
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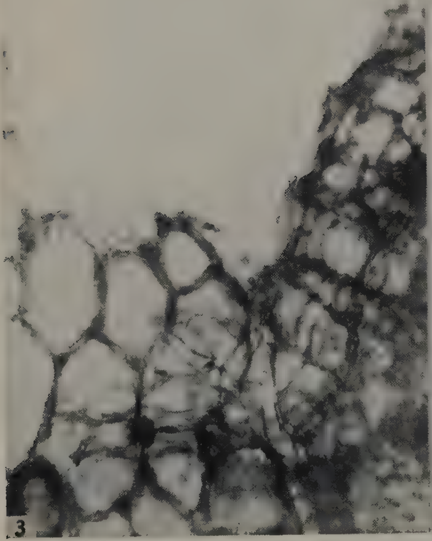
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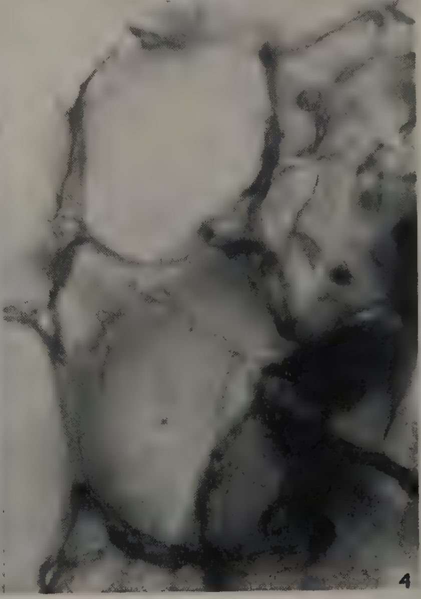
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Trans. Brit. mycol. Soc. 42 (1), 67–71 (1959).

A PURE CULTURE OF *CHYTRIOMYCES AUREUS* KARLING

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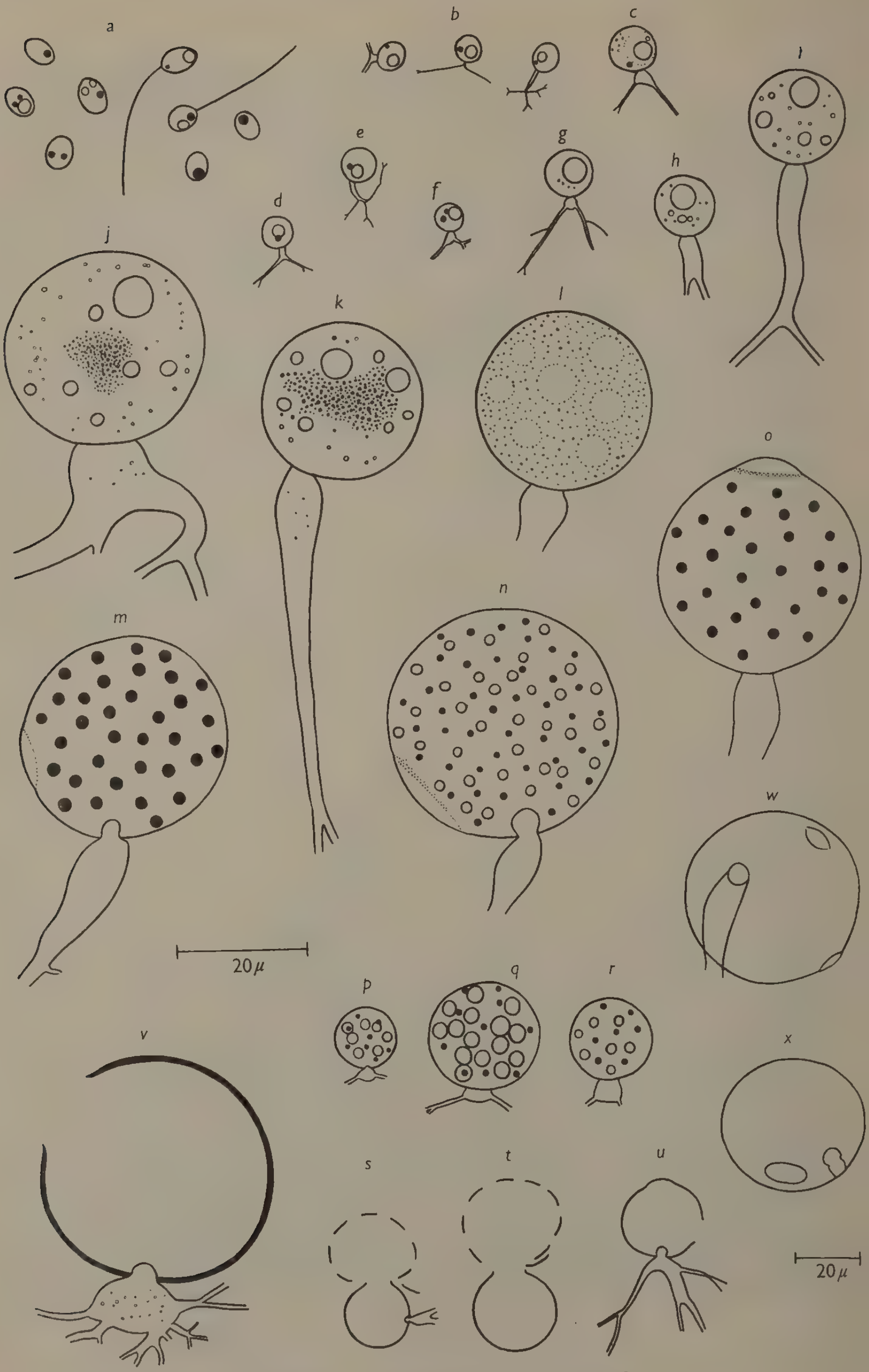
(With Plate 5 and 1 Text-figure)

Chytriomyces aureus Karling is reported from Britain for the first time. The zoospores of the strain studied were noteworthy in that they often possessed both a pigmented and a hyaline oil globule. Antibiotics were used to obtain a pure culture on YpSs agar. In culture there was prolific growth on chitin and a fair amount of growth on keratin. There was growth in liquid medium provided yeast extract was present. Glucose was utilized as a carbohydrate source. Decomposition of cellulose could not be demonstrated.

Saprophytic chytrids from the littoral zone of Windermere and Esthwaite Water (Lake District) are being investigated. One method used in this work is to leave submerged celluloid traps containing fragments of newspaper and cellophane in suitable positions in the lake, and recover them after 2 or 3 weeks. The cellulosic material is then placed in small beakers, covered with distilled water, and warmed to 25° C. Normally this procedure results in the dehiscence of any mature chytrid sporangia present on the substratum, and motile zoospores accumulate in large numbers at the surface meniscus. These spores are then looped off on to quarter strength Emerson's YpSs tellurite agar or maize tellurite agar and incubated at 25° C. The majority of the spores give rise to polycentric chytrid thalli, and monocentric forms only appear occasionally. One monocentric chytrid which was obtained in this way from Windermere has been studied in detail. Since only a single thallus was found on the agar this could have originated from a stray spore in the water rather than from growth on the cellulose. Subsequent work reported below indicated that this was the case.

The thallus was transferred to a slope of quarter strength YpSs medium solidified with 0·7 % agar. At this concentration of agar a good deal of liquid accumulates in the bottom of the tube. After the thallus had been in this liquid for several days, numerous colonies were visible to the naked eye on the submerged agar. The liquid was then rolled over the surface of the slope daily, and after a week the entire surface of the agar was covered with thalli. This accumulation culture was not pure, and to eliminate bacteria a mixed penicillin streptomycin solution was used on a zoospore streak, following the procedure of Vishniac (1956). Frequent subculturing was necessary to maintain viable cultures. Portions of a culture were transferred to flasks of lake water containing: (i) decalcified shrimp chitin, (ii) untreated snake skin, and (iii) P.T. 300 nonwaterproof cellophane. All flasks and their contents were sterilized before



Text-fig. 1. See opposite page for legend inoculation. There was prolific growth on the chitin (pH 6·8), fair growth on the snake skin (pH 7·2) and none on the cellophane. On chitin numerous holes appeared as the result of chytrid growth and this suggested that the fungus was chitinophilic.

There was good agreement between my organism and *Chytriomyces aureus* Karling (1945), but some further points were noted. Zoospores were oval, 4·5–5·5 × 3·5–4 μ with a posterior flagellum 18–20 μ (Text-fig. 1 *a*; Pl. 5, figs. 3, 4). In addition to a golden red oil globule, usually situated near the flagellum insertion, there was often a hyaline globule. The golden red globule was 0·5–1·5 μ diam., and the hyaline globule 1–2 μ diam. Two pigmented or two hyaline globules occasionally appeared in a single spore. In liquid culture (see below) zoospores of all the types illustrated were recovered at various times. When both pigmented and hyaline globules were present they could be clearly distinguished in mature sporangia prior to dehiscence (Text-fig. 1 *n*, *p–r*). After encystment the zoospore expanded evenly and in development the pigmented globule eventually disappeared (Text-fig. 1 *b–i*). Pigment only reappeared in a late stage of development, first centrally (Text-fig. 1 *j*, *k*), and then evenly distributed through the sporangium (Text-fig. 1 *l*). It was noticeable that cultures on agar always appeared white to the naked eye, indicating that the amount of pigment present in a mass of thalli is small.

At maturity the sporangia were 10–34 μ diam. on YpSs agar, 14–43 μ diam. on chitin, and 12–58 μ diam. on keratin. The operculum was 5–14 μ diam., very inconspicuous, and its position bore no constant relation to the insertion of the rhizoidal system on the sporangium (Text-fig. 1 *m–o*, *s*, *u*, *v*, *x*; Plate 5, fig. 5). There was evidence that two opercula occurred occasionally (Text-fig. 1 *u*, *w*). An apophysis, 5–12 μ diam., was invariably present on thalli grown in liquid culture, but on the solid substrata chitin and keratin, and on the surface of agar, there was often a single long rhizoidal axis instead (Text-fig. 1 *i*, *k*, *m*).

On dehiscence the delicate operculum was detached. There was vigorous collective swarming both inside the sporangium and in a vesicle outside before the zoospores dispersed (Text-fig. 1 *s*, *t*). Detached opercula were rarely seen after dehiscence. Resting spores were not observed during the course of this work.

The question arose as to whether my strain of *C. aureus* could be induced to attack cellulosic materials in addition to chitin, since Karling suggested that this organism had both these capacities, although it was primarily

Text-fig. 1. *Chytriomyces aureus* Karling; *a*, zoospores; *b–f*, young thalli in which the pigmented oil globule of the zoospore is still visible; *g–i*, young thalli with no pigment visible; *j*, *k*, young thalli with pigment appearing centrally; *l*, young thallus with vacuoles and evenly distributed pigment; *m–r*, mature sporangia; *s*, *t*, dehiscence in two sporangia. In each case a shed operculum is visible to the right of the exit orifice. The outline of the swarm-vesicle is indicated by an interrupted line; *u*, a dehisced sporangium which has shed one operculum. A second operculum situated opposite the insertion of the rhizoidal system was not displaced; *v–x*, dehisced sporangia. In *w* there were two exit orifices, and part of the rhizoidal system is drawn in on the left. The sporangium *x* is detached from its rhizoidal system, but part of this persists as a knob projecting inwards to the right of the exit orifice. In all drawings pigmented oil globules are rendered in black, hyaline oil globules are drawn in outline. *s–u*, *w*, *x* are at the scale below *x*.

chitinophilic. Pure culture studies by Crasemann (1954) on a species of *Chytridium* showed that this organism could attack chitin but not cellulose.

Accordingly, some work was carried out in liquid culture (Pl. 5, figs 1, 2). Preliminary trials suggested that the fungus could grow in the pH range 5·2–7·8; but since pH drift was considerable at the weak phosphate concentration used, especially when glucose was present, it was decided to correct this daily by the addition of sterile N/10 KOH or HCl. 0·25 ml. of a 0·04 % (w/v) solution of Bromo Thymol Blue was added to each 50 ml. of medium, and pH was maintained at 6·8. The liquid medium base was: 0·26 g. $MgSO_4 . 7H_2O$, 20 μg. thiamine, 4 μg. biotin, 20 ml. Sørensen's buffer per l.

0·1 p.p.m. Cu and Zn were added as $CuSO_4$ and $ZnSO_4$, respectively, and 0·5 p.p.m. Mn and Fe as $MnCl_2$ and $FeCl_3$. The medium was dispensed in 50 ml. amounts in conical flasks and inoculated with a tiny piece of a culture on agar. There was no growth in this medium when 0·5 % glucose and ammonium tartrate or sodium nitrate were added, and it is not certain if inorganic N sources can be utilized for growth. The addition of 0·1 % yeast extract only, resulted in fair growth, but the addition of 0·5 % glucose as well gave greatly enhanced yields (visual assessment) and larger sporangia. These were up to 70 μ diam. while with yeast extract only they were no larger than those on YpSs agar. Yields in the liquid medium base with the addition of (i) cellophane + yeast extract, and (ii) yeast extract only, were compared by computing total cell volume (excluding apophysis and rhizoids) present in one field of a haemocytometer counting chamber. No appreciable difference could be detected in the amount of growth recorded under these two conditions. The cellophane appeared unattacked, and any thalli adhering were easily washed off with a gentle stream of liquid. There was also no decomposition of cellophane in the presence of yeast extract at pH 7·8, or in the presence of yeast extract + ammonium tartrate or yeast extract + sodium nitrate at pH 6·8, although in each case growth took place in the liquid. In a further experiment, to the liquid medium base + yeast extract was added 0·25 % and 0·5 % glucose, and casamino acids and peptone at 0·005 % and 0·01 % N levels. These additions of glucose and organic N were made alone and in all possible combinations. To each flask was added cellophane, filter-paper, lens tissue, and Kleenex tissue. There was good growth in all the flasks, but the cellulosic material remained unattacked.

It seems clear, therefore, that under the conditions stated, the strain of *C. aureus* studied had no capacity to digest cellulose, and the term chitinophilic can be both conveniently and appropriately applied to it.

My thanks are due to Prof. C. T. Ingold for kindly reading and commenting on the manuscript.

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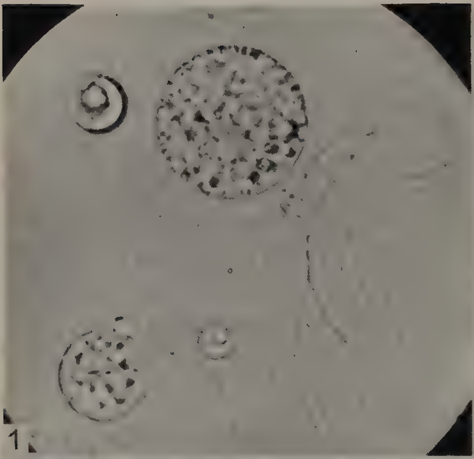
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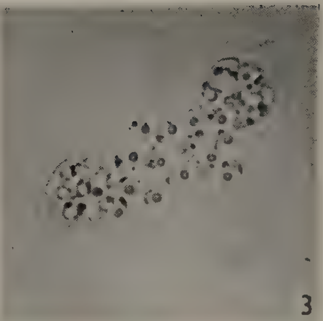
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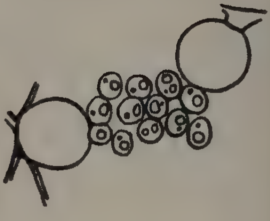
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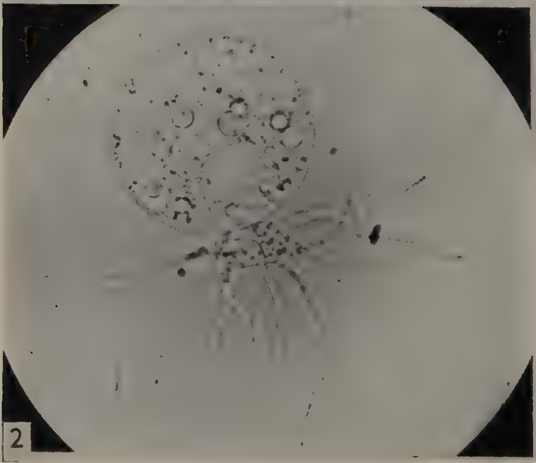
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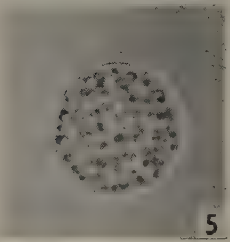
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EXPLANATION OF PLATE 5

Chytriomyces aureus Karling. (All × 850.)

Figs. 1, 2. Young thalli growing in glucose peptone liquid medium.

Fig. 3. Two sporangia on YpSs agar have released zoospores. Both hyaline and pigmented globules are present in the spores.

Fig. 4. Diagrammatic explanation of Fig. 3. Hyaline globules are drawn in outline. Pigmented globules are rendered in black, where they were clearly observed.

Fig. 5. A small mature sporangium on YpSs agar. The rhizoidal system is not visible. The operculum is at the top.

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ARACHNIOTUS INDICUS SP.NOV.

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(With 2 Text-figures)

Arachniotus indicus sp.nov. from the soil of paddy fields of State Agricultural Farm, Chinsurah, India, is described. *A. indicus* var. *major* var.nov. differing from the type species in the size of the cleistothecium, coloration and structure of the wall of the peridium is also described.

Arachniotus indicus sp.nov. (Fig. 1)

Coloniae ad 25° C. late diffusae in agar dextroso solanaceo. Hyphae aereae primo floccosae, tum intextae, suntque hyalinae, angustissimae, 0·5–1·8 μ diam., ramosae, septatae, cleistothecia in agar dextroso solanaceo ad 25° C. primo apparent post 96–120 horas ut corpora minuta luteola pallida, suntque irregulariter dispersa supra seriem superficialem mycelii, sed arctius aggregata ad medium.

Cleistothecia sunt globosa vel subglobosa, diametrientia 162·5–520 μ, pallide aurantiaco-lutea, sessilia, absque ullis appendicibus. Parietes cleistothecii reticulati, constantes e hyphis quae sunt satis dense decussate dispositae efformantes peridium imperfectum. Parietes peridiales tenues, constantes e singulis vel binis seriebus cellularum, sed sunt lenti et applani sub pressione sed haud disrupti. Mycelium peridii tenuibus parietibus praeditum, ramosum, aurantiaco-luteum, sed distinctum a mycelio in cleistotheciis, quod a hyphis vegetativis non differt.

Asci surgentes e mycelio ramoso in cleistotheciis, orbiculares vel tenuiter ovati, lutei in massa, tenuibus atque pallide aurantiaco-luteis parietibus praediti, 7·8–13 × 7·0–9·3 μ, mediet. 9·8–7·8 μ, deliquescentes ad maturitatem. Ascosporae 8 in singulis ascis, unicellulatae, crassis parietibus praeditae, pallide aurantiaco-luteae, ovatae vel globosae, 3·2–4·0 × 2·8–3·6 μ mediet. 3·6 × 3·2 μ.

Typus lectus in humo in orizetis in State Agricultural Farm, in loco Chinsurah, in provincia West Bengal in India (Herb. I.M.I. 57850).

In the course of study of soil fungi of paddy fields at the State Agricultural Farm, Chinsurah (West Bengal, India), a species of *Arachniotus* was isolated. A study of the literature failed to reveal any description that matched this fungus, which differed from the other species of *Arachniotus*, and by reference to the Commonwealth Mycological Institute it was found that the organism could not be matched with any specimens in Herb. I.M.I. or with any described species (personal communication from Director, Commonwealth Mycological Institute, Kew), so it is described as new.

Colonies on potato-dextrose agar at 25° C. are broadly spreading. Aerial hyphae are floccose at first, but later matted. Aerial hyphae are hyaline, very narrow 0·5–1·8 μ diam., branched, septate. Cleistothecia begin to appear within 96–120 hr. on potato-dextrose agar at 25° C. as minute, faint, yellowish bodies, which are irregularly scattered over the surface layer of the mycelia, but more closely arranged near the centre.

Cleistothecia are globose to subglobose, light orange yellow, sessile, without any appendages 162·5–520 μ diam. Wall of cleistothecium web-like, composed of hyphae which are comparatively densely arranged in a cross-wise manner forming an imperfect peridium. Peridial wall thin, composed of one to two layers of cells, but tough, becoming flattened under pressure but does not break. Peridial mycelium thin-walled, branched, orange-yellow in colour and distinct from the mycelium inside the cleistothecium which is indistinguishable from the vegetative hyphae.

Asci developing from the branching mycelium within the cleistothecia, round to slightly oval, light yellow in mass, thin-walled, wall light orange-yellow, 7·8–13 × 7·0–9·3 (av. 9·3 × 7·8) μ, deliquescing when mature. Ascospores eight per ascus, 1-celled, thick-walled, light orange-yellow, oval to globose, 3·2–4·0 × 2·8–3·6 (av. 3·6 × 3·2) μ.

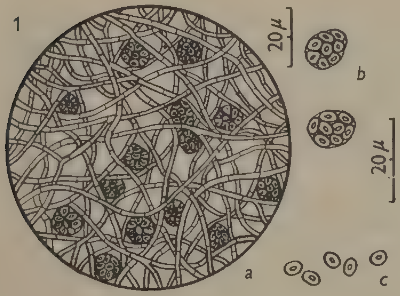

Fig. 1

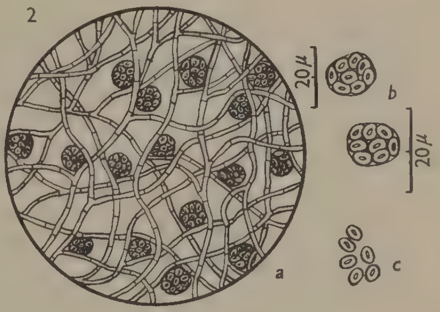

Fig. 2

Fig. 1. *Arachniotus indicus* sp.nov. *a*, cleistothecium; *b*, asci with ascospores; *c*, ascospores.

Fig. 2. *Arachniotus indicus* Chattopadhyay & Das Gupta var. *major* var.nov. *a*, cleistothecium; *b*, asci with ascospores; *c*, ascospores.

In course of examination of different isolates of *Arachniotus indicus*, an isolate was found which was similar to *A. indicus* in the shape and size of the ascospores, but was observed to differ from the type species in the size of the cleistothecium, coloration and structure of the peridial wall. Cleistothecia in this case measure from 227·0 μ to 1·0 mm. The peridial wall is formed of mycelial threads, which are comparatively loosely arranged and cleisothecia easily flatten under pressure. Cleistothecia are bright yellow in colour at first, but later turn brown with age. Appearance of cleistothecia is rather irregular, and not within a definite time period as in the typical case. So it has been considered to be a new variety of *A. indicus*.

Arachniotus indicus Chattopadhyay & Das Gupta var. **major** var.nov. (Fig. 2)

Differt a typo cleistotheciis majoribus, diametrientibus 227·0μ–1·0 mm., charactere mycelii peridialis, quod sat faciliter disrumpitur, estque fusce, brunneum colore. Cleistothecia sunt pallide brunnea ad maturitatem. Fructificationes, contra ac in typo, inconstantes sunt, ideoque mycelium saepe sterile permanet.

Typus lectus in humo in orizetis in State Agricultural Farm, in loco Chinsurah, in provincia West Bengal in India (Herb. I.M.I. 57851).

We express our thanks to Dr J. C. F. Hopkins, Director, Commonwealth Mycological Institute, Kew, for his help in identification, and to Rev. Father Dr H. Santapau, St Xavier's College, Bombay, for rendering the description into Latin.

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SOME ZOOPAGALES FROM KENYA

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(With 11 Text-figures)

An account is given of Zoopagales occurring in Kenya Colony (East Africa). Seventeen species and varieties are recorded, of which *Acaulopage aristata*, *A. retusa* and *Cochlonema pygmaea* are described as new.

Since the Zoopagales were first recorded by Drechsler (1935*a*), seventy-four species have been described, most from North America, but a few from England. A number of the American species are also known to occur in Britain, though they often vary in some regular way from the type descriptions which necessitates their identification as ecotypes or even as varieties.

Examples of this type are *Stylopage araea* Drechsler var. *magna* Peach & Juniper (1955) in which the conidial size range lies appreciably above that of the American type variety (Drechsler, 1935*b*), and *Acaulopage ischnospora* Drechsler (1947*b*), fairly common in England but differing from the type description in the relatively small percentage of conidia with evacuated apices (Duddington, 1951). On the other hand, especially with some filamentous spored *Acaulopage* specimens, it is often impossible to place them with any conviction in any of the described species (Duddington, 1956), and here perhaps, where the type descriptions are rather close, we are dealing with fewer but more variable species than at present recognized.

So far, with the one exception of *Stylopage hadra* Drechsler (1935*a*) from Hawaii (Linford & Oliveira, 1938), no reports of the Zoopagales have appeared outside Britain or America, yet knowledge of the variation shown by these fungi elsewhere would be of value in aiding our evaluation of the species problem. The author is at present studying these fungi from several geographical regions. This first account covers Zoopagales from Kenya.

MATERIALS AND METHODS

Samples were collected from the Rift Valley (Nakuru) and Mount Kenya (Kikuyu) Provinces of Kenya Colony, East Africa, and also from Nairobi. Most of this area lies at a high altitude and consequently has a moderate temperate climate with a fair amount of rain. Collections were made from various habitats of which a representative sample is listed in Table 1. It is noticed that best results were obtained from damp material containing a high percentage of decaying organic matter.

Samples were plated within 4 days of collection on rabbit-dung agar (R.D.A.) (2 % agar solution tinted a pale straw colour by the addition of filtrate from boiled rabbit dung). Maize-meal agar was employed on a few occasions, but R.D.A. gave consistently better results through the weaker growth of saprophytic fungi. No apparent or significant difference was shown by *Stylopage rhabdospora* Drechsler or *Acaulopage tetraceros* Drechsler on the two different media.

Table 1

Locality	Sample	No. of species
Nakuru	Cultivated vegetable garden	6
Nakuru	Non-cultivated land with sparse vegetation	0
Mount Kenya	Decaying surface litter from damp woodland	5
Mount Kenya	Soil from same situation, but 4–6 in. below surface	0
Naivasha	Debris round base of tree	4
Naivasha	Stony ground, sparse scrub	0
Naivasha	Soil from margin of lake	2

Observations on individual fungi were often extended over 4 months; sometimes from subcultures, but more usually from the original plate, as the Zoopagales are generally unreliable in subculturing. All drawings were made from living material by aid of a camera lucida.

A few fungi too ephemeral for full description to be obtained are omitted from the following list.

LIST OF SPECIES

Acaulopage aristata sp.nov. (Fig. 1)

This fungus appeared in samples obtained from two separate sites at Nakuru: a garden compost heap, and cultivated ground (flower garden). On both occasions it proved a slow but persistent grower and though kept under observation for 4 months showed no evidence of sexual reproduction.

The mycelium was sparse and rather delicate with hyphae 1·0–1·5 μ wide. Small amoebae (20–30 μ across) adhering to the mycelium were invaded by dichotomously branched haustoria, with lobes about 5 μ long and about 0·7–1·0 μ wide, borne on a thin stalk 3–5 × *c.* 0·5–0·8 μ (Fig. 1 *a*).

Conidia borne on short sterigmata 1·5–2·0 μ high, developed at intervals of 2–30 μ from straight, sparingly branched, prostrate hyphae. Where occurring at longer intervals the conidiophores proliferated to give 3, and occasionally 4, conidia, which on account of their close spacing on the conidiophore tip, formed a divergent group (Fig. 1 *b*) similar in appearance to *Stylopage cephalote*.

The conidium (Fig. 1 *c*) at maturity consisted of a living elongate-ellipsoidal cell, 13·0–32 (mostly 15–24) × 2·0–4·2 (mostly 3) μ with an empty distal appendage, 4·0–21·0 (mostly 14–17) × 0·7–1·0 μ at the base, tapering gradually to a narrow, rounded tip. Occasional conidia possessed a forked tip; this involved 4 % of the conidia in one sample, but 15 % in the other (Fig. 1 *d*).

Drechsler has described a number of species which bear a superficial resemblance to this fungus. Thus *A. gomphoclada* (Drechsler, 1942) has shorter and much narrower conidia (basal living part 11–22 × 1·3–1·8 μ) and also shows sexual reproduction with distinctive lateral conjugation; *A. cercospora* (Drechsler, 1936) has much shorter conidia (basal living part 7–15 × 2·2–3·6 μ) with longer conidiophores; *A. marantica* (Drechsler, 1939*a*) has longer conidia (living part 33–52 × 2·4–3·1 μ), and also a small basal evacuated portion in addition to the empty apex; and finally *A. rhicnospora* (Drechsler, 1935*b*) a delicate fungus with narrower conidia (total length 20–55 μ, 1·5–2·0 μ wide).

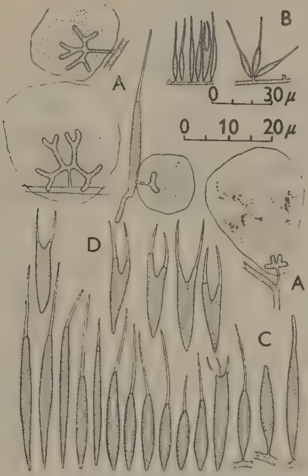

Fig. 1. *Acaulopage aristata* sp.nov. A, Haustoria; B (drawn to smaller scale), conidiophores bearing several conidia; C, D, conidia.

This fungus cannot be described as a variety of any of the above species, differing from each in several qualities, but is considered sufficiently distinctive to warrant specific rank, the specific epithet *aristata* referring to the awned conidia.

Mycelium sparsum, hyphis continuis, hyalinis, 1·0–1·5 μ crassis, haustoria pedicellata evolventibus; pedicello 3–5 μ longo, 0·5–0·8 μ crasso; ramulis dichotomis 5 μ longis, 0·7–1·0 μ crassis. Conidia solitaria, interdum usque 4, ex sterigmatibus erectis circiter 2 μ altis orta; cellula viventi protoplasmatis repleta; elongata-ellipsoidea, 13–32 μ longa, 2·0–4·2 μ crassa, appendicem apice ferente; appendice vacua, 4–21 μ longa, 0·7–1·0 μ crassa, interdum bifurcata.

Zygosporae ignotae. Habitat in humo, amoebas 20–30 μ latas capiens et consumens, prope Nakuru.

ACAULOPAGE DICHOTOMA Drechsler (Fig. 2)

This distinctive fungus occurred with *A. dichotoma* var. *longa* in a pond at Nakuru. It agreed closely in conidial shape and size with the type species (Drechsler, 1945) and British examples. The conidia (Fig. 2*c*) were 1–3 times dichotomously branched with distal evacuated branch apices, borne rather scantily on a very sparse mycelium.

ACAULOPAGE ISCHNOSPORA Drechsler (Fig. 4)

The species of *Acaulopage* with filamentous conidia are often difficult to identify, as the named species have rather similar spore size ranges, and are separated mainly on slight differences in conidial shape. The presence of sexual stages sometimes facilitates identification.

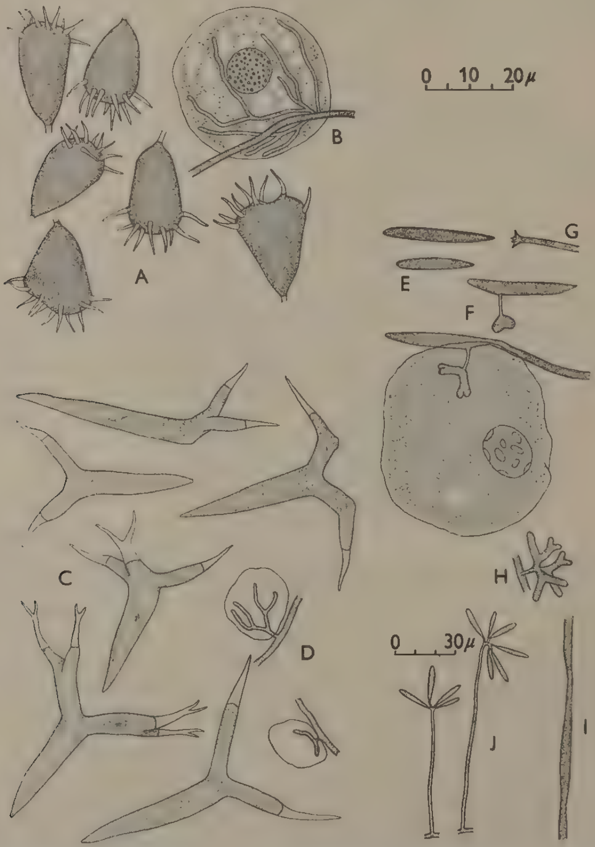

Fig. 2. *Acaulopage lophospora* Drechsler (A, B). A, Conidia; B, haustorium. *A. dichotoma* Drechsler (C, D). C, Conidia; D, haustoria. *Stylopage cephalote* Drechsler (E–J). E, Conidia; F, germinating conidia with haustoria; G, tip of conidiophore; H, fully developed haustorium; I, irregular hypha, common in this species, and frequent in the Zoopagales; J (drawn to smaller scale), conidiophores.

The fungus under discussion is considered sufficiently close to *A. ischnospora* (Drechsler, 1947) to be so listed though the size range of the conidia ($37{\cdot}0–69{\cdot}5 \times 1{\cdot}5–2{\cdot}0\,\mu$) was lower than Drechsler's (about $54–90 \times 1{\cdot}6–2{\cdot}0\,\mu$). Also, the conidia showed a more pronounced basal taper, and only about 50 % had evacuated tips.

British examples I have observed and allocated to the same species have a similar conidial size range, and only 5–10 % with empty tips, but these can be positively identified by Drechsler's drawings of the sexual stages. Unfortunately the Kenya material did not produce zygospores.

The conidia of this fungus were borne singly, but later proliferation of the short pedicel regularly produced two and later three conidia—an approach to the *Stylopage* condition (Fig. 4*b*).

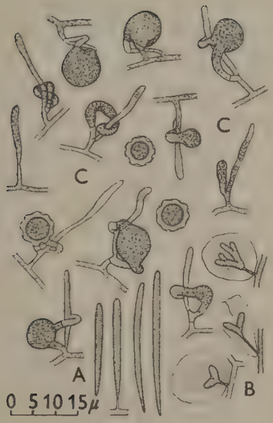

Fig. 3

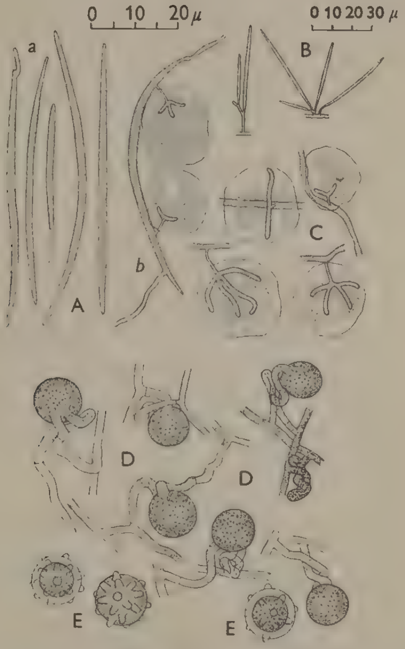

Fig. 4

Fig. 3. *Acaulopage retusa* sp.nov. (A–C). A, Conidia; B, haustoria; C, stages in zygospore formation.

Fig. 4. *Acaulopage ischnospora* Drechsler (A–C). A, Conidia, germinating at '*a*' and '*b*'; B (drawn to smaller scale), conidiophores; C, haustoria. *A. tetraceros* Drechsler (D, E). D, stages in zygosporangium formation; E, mature zygospores.

ACAULOPAGE LOPHOSPORA Drechsler (Fig. 2)

Two of the fungi obtained were readily identified as *A. lophospora*, being very close to the type description (Drechsler, 1946) and British examples, in size and form. The conidia were 17·5–28·5 × 9·7–15·3μ, with 12–16 evacuated distal appendages, 4·0–9·0μ long. No sexual stages were observed.

Acaulopage retusa sp.nov. (Fig. 3)

Mycelium sparsum, hyphis continuis, hyalinis, 1·0–1·5μ crassis, haustoria pedicellata evolventibus; ramulis dichotomis 1·6–2·0μ crassis. Conidia solitaria, ex sterigmatibus erectis 2μ altis orta, filiformia, apice abrupte rotundata; ad basi acita attenuata; 21–33μ longa, 1·5μ crassa. Zygosporangia ex copulatione hypharum brevium uterque ex una hypha crassa lateralis emergens; maturitatae nonnihil collbentia. Zygosporae 6·8–8·0μ diam., loculo 4·0–5·4μ, verrucis ornata. Habitat in humo, amoebas 8–15μ latas capiens et consumens, prope Nakuru.

This fungus was obtained from a vegetable plot at Nakuru.

The mycelium of clear hyphae 1·0–1·5 μ wide produced stalked haustoria, the haustorial lobes 1·6–2·0 μ wide in small amoebae 8–15 μ across.

The filiform conidia had abruptly rounded tips tapering gradually to a narrow base; 21–33 (mostly 24–30) × 1·5 μ. They were borne singly on short sterigmata about 2 μ high arising from prostrate hyphae distinct from the nutritive hyphae.

Conjugation commenced soon after the first appearance of the conidia. The zygosporangia resulted from the distal fusion of two short, usually club-shaped, lateral hyphae. At maturity the zygosporangium wall collapsed around the thick-walled zygospore ornamented with rounded processes, 8 or 9 of which were visible in profile. The overall diameter of the zygospore was 6·8–8·0 (mostly about 7·5) μ with a protoplast 4·0–5·4 μ across.

The only species with which this fungus might be confused is *A. baculispora* Drechsler (1948), where also the conidia have abruptly rounded tips. Here, however, the conidia are almost cylindrical, tapering only slightly to the somewhat rounded base, and larger (21–62 × 1·8–2·1 μ). The zygospores of *A. baculispora* are appreciably larger, being 6–11 μ across.

This fungus appears distinct in conidial shape and size range from other published accounts and has characteristic sexual stages. It is therefore proposed as a new species, the epithet *retusa* referring to the obtuse conidium apex.

Acaulopage tetraceros Drechsler (Fig. 5)

This is apparently the commonest species in the regions studied, occurring in almost all of the samples investigated. Perhaps this fungus is favoured by the particular culture methods; it is certainly more readily and successfully subcultured than is general with the Zoopagales. *A. tetraceros* also occurs relatively frequently in England.

Kenya specimens agree closely with Drechsler's (1935*b*) description and also with English stock. The conidia were 16·0–25·5 μ long (excluding the basal appendage), 6·5–11·3 μ wide and have 2–8 (usually 4) empty distal appendages, 6·5–21·0 μ long. However, sexual stages, previously not recorded, have occurred in fungi obtained from Nakuru and Naivasha.

Sexual activity ensued 7–14 days after conidial formation and persisted for several weeks. The zygospores were found generally below the surface of the medium at the point of junction of hyphae about 2·0 μ wide.

The mature zygospore wall had 16–22 prominent protuberances with rounded tips, the zygosporangium wall not being separately distinguishable. The overall diameter was 9·8–13·8 (mostly 12·0–13·0) μ or 9·2–12·0 μ excluding the processes. The central protoplast had a diameter of 6·8–9·3 μ surrounded by a wall 1·2–1·8 μ thick.

Acaulopage tetraceros Drechsler var. *longa* Jones & Peach

This fungus has appeared twice in Kenya soil samples; from a pond at Nakuru, and from wet debris in dense woodland on Mount Kenya; these habitats according well with its English stations—ponds, streams and wet ground.

The Kenya examples agreed closely with English forms; the spores were 24–36 (excluding basal appendage) × 4·8–8·7 μ, bearing 2–4 (mostly 3) empty distal appendages 8–35 μ long.

Sexual reproduction, observed in the type variety, did not appear in these samples.

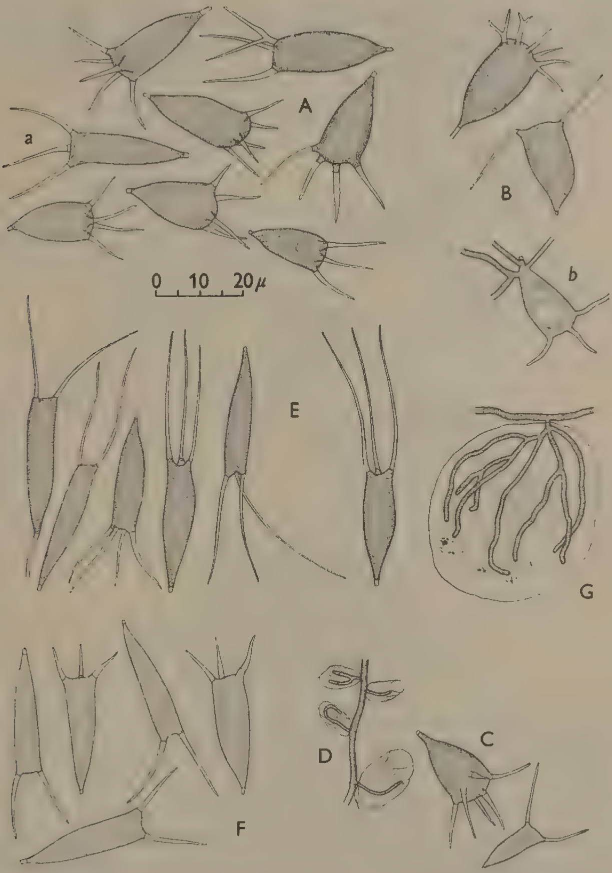

Fig. 5. *Acaulopage tetraceros* Drechsler (A–D). A–C, Range of conidial shape from three separate fungi. The conidium at *a* is an extreme form resembling *A. tetraceros* var. *longa*. The conidium at *b* has germinated. D, Haustoria of the simple type produced in small amoebae. *A. tetraceros* Drechsler var. *longa* Jones & Peach (E–G). E, F, Range of conidia from two separate fungi, with distal processes of different lengths; G, haustorium of fungus at E.

Cochlonema pygmaea sp.nov. (Fig. 6)

Hyphae nutritae 2·5–4·0 μ crassae, simplices, in 1½–2 μ spiras convolutae, intra corpus amoebarum viventum. Conidia fusoidea, membranis non regulis 3·5–6·0 μ longa, 1·2–1·5 μ crassa, ex hyphis erectis, aeris. Habitat in humo amoebas 25 μ latas capiens et consumens, prope Nakuru.

About 7 weeks after inoculating a series of plates with material from a Nakuru compost heap, a few dead amoebae (about 25 μ across) were found parasitized by a small *Cochlonema* type thallus. A few weeks later large-scale infection was immediately obvious from the many erect chains of short conidia arising from the dead hosts. At this stage, multiple infection was common, many amoebae containing 5 or 6 thalli which were consequently appreciably smaller when mature (though without effect on spore size).

Infection of the host was apparently by ingestion but although actual germination of the spores was not observed, it is apparently terminal since the thallus was always attached to one end of the old spore.

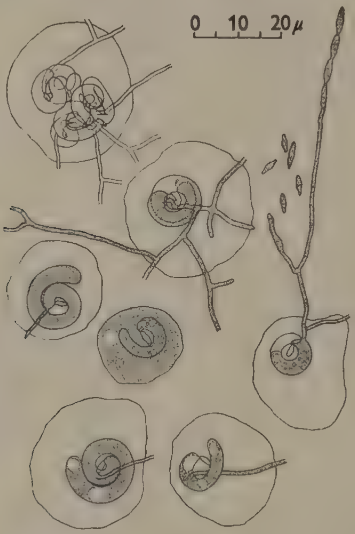

Fig. 6. *Cochlonema pygmaea* sp.nov.

The mature thallus was always simple—never branched—and consisted of a single hypha coiled in 1½ to 2 turns, with a maximum diameter of from 2·5 to 4·0μ, but mostly about 3·5μ. The entire thallus had an overall width of 9–16μ. When fully grown, a single hypha, 0·8–1·3μ wide, appeared from the proximal end of the thallus (i.e. near the original germ tube) penetrated the membrane of the dead animal and divided to give 2–8 (usually 2–3) erect branches bearing chains of 30–47 conidia. These aerial hyphae, 180–240μ high, afforded a ready means of locating the fungus and assessing its abundance, persisting for 7–10 days before collapsing to the substratum and breaking up into separate spores.

The conidia were spindle-shaped with rounded ends and irregular walls: 3·5–6·0 × about 1·2–1·5μ, the longer conidia being relatively few in number and positioned to the base of the chains.

No sexual reproduction occurred at any time, although multiple infection later became frequent and presumably gave ample opportunity from the close proximity of different thalli.

This fungus resembles *Cochlonema agamum* Drechsler (1946) in the general appearance of the thallus and conidia, but here the thalloid hyphae are 4–13μ diam. and the spores 6–11 × 1·5–2·5μ, all above the ranges of the fungus under consideration. Furthermore, the thallus in *C. agamum* may show one or two dichotomies in the endozoic thallus, and apparently readily forms ayzgospores. Confusion here is therefore unlikely, and in fact this species is most readily separated from all other members of the genus by its small size, hence the specific epithet of the proposed binominal for this new species.

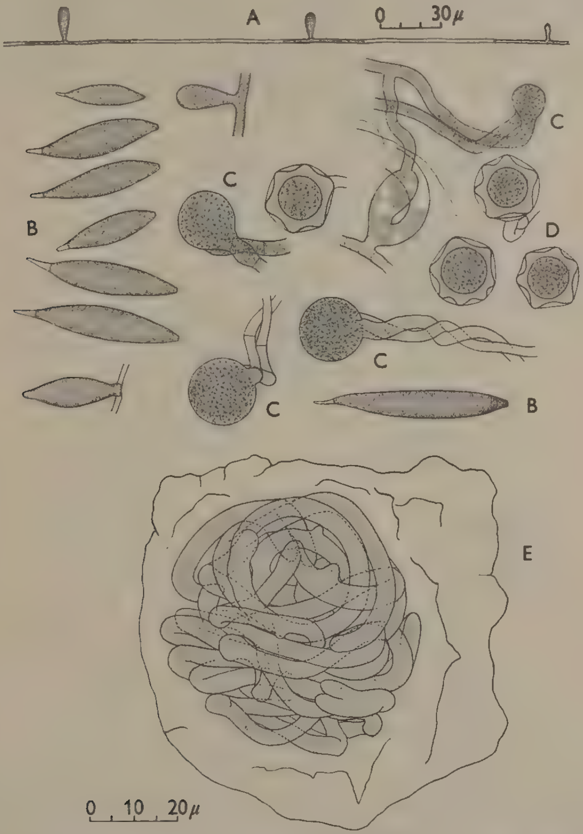

Fig. 7. *Endocochlus gigas* Drechsler. A (drawn to smaller scale), Conidia arising from prostrate hypha; B, conidia; C, zygosporangia; D, zygospores; E, mature thallus.

ENDOCOCHLUS GIGAS Drechsler (Fig. 7)

This record is of interest as the first species of *Endocochlus* noted outside America, all the more remarkable from the especially large size of *E. gigas* which was one of the earlier Zoopagales to be described by Drechsler (1936). It was found on platings of garden soil from Nakuru; differences from the type description were but slight.

The thallus was frequently branched—in the larger thalli which formed a large knot about 60 μ across it was impossible to ascertain the degree of branching—with hyphae 4·5–7·5 μ diam. The conidia were conspicuous, being borne at intervals of 120–190 μ on sparingly branched prostrate hyphae 2·5–3·0 μ wide extending from 2·0 to 3·4 mm. from the dead host.

They were a little wider than Drechsler's, having a total length of 21–44 μ and width of 5·4–7·5 μ.

Zygosporangia were readily formed, frequently below the surface of the medium, at the point of junction of two conjugating hyphae, and not on a short hyphal extension from this region as described in America. Perhaps as a consequence of their larger size zygosporangium and zygospore formation was more protracted than in the usual run of Zoopagales, the large thick-walled zygospores (13·5–16·5 μ wide) normally requiring 7 days for development to maturity, from the first fusion of the gametangia. The enclosed protoplast measured 9·0–10·5 μ diam.

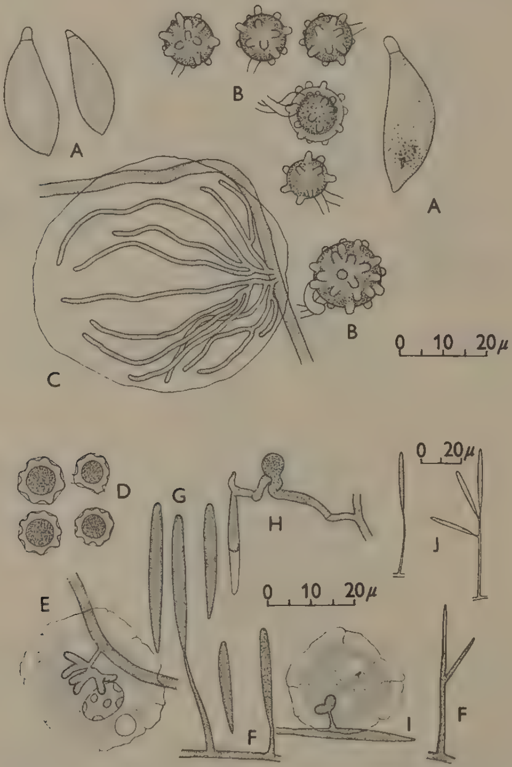

Fig. 8. *Stylopage rhynchospora* Drechsler (A–C). A, Conidia; B, zygospores; C, haustoria. *S. rhabdoides* Drechsler (D–J). D, Zygospores; E, haustoria; F, conidiophores; G, conidia; H, zygosporangium formation; I, haustorium from a germinating conidium; J (drawn to smaller scale), conidiophores.

STYLOPAGE CEPHALOTE Drechsler (Fig. 2)

This fungus differed from the type description (Drechsler, 1938) in the rather larger conidial size—19–28 × 2·4–3·0 μ—in this respect agreeing with English examples. This trend to the larger spore size of *S. rhabdospora* Drechsler is also seen in the arrangement of the conidia on the conidiophore, where in most examples of Kenya and English stock obvious proliferation of the conidiophore tip is apparent rather than the expanded head in the American drawings. Sexual behaviour was normal.

Stylopage haploe Drechsler and *Stylopage lepte* Drechsler (Fig. 9)

Duddington (1955) described *S. haploe* from England and pointed out its dissimilarities from the type description (Drechsler, 1935*b*). Kenya specimens also show a proliferation of the conidiophores, so that while most of the conidia are borne singly, (5–10 %) of the conidiophores proliferate to produce 2, 3, or even up to 8 conidia. Otherwise the spore size range is that of Drechsler and the fungus is to all intents similar to Drechsler's description.

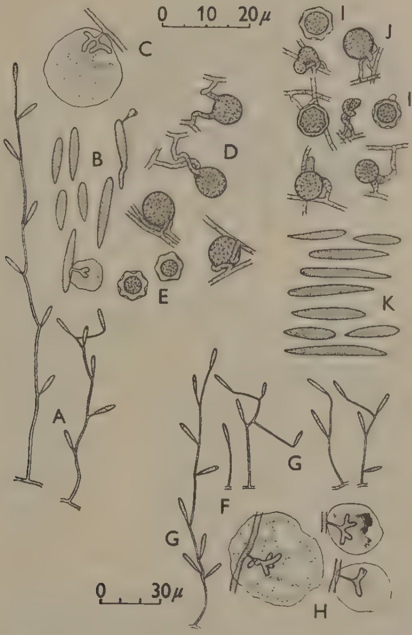

Fig. 9. *Stylopage lepte* Drechsler (A–E). A (drawn to smaller scale), Conidiophores; B, conidia; C, haustoria; D, zygosporangia; E, mature zygospores. *S. haploe* Drechsler (F–J). F (drawn to smaller scale), usual form of conidiophore; G (smaller scale), conidiophores which have proliferated and resemble those of *S. lepte*; H, haustoria; I, zygosporangia; J, mature zygospores; K, conidia.

S. lepte (Drechsler, 1935*b*) has been recorded on several occasions from England and Kenya, bearing regularly 6 or more conidia per conidiophore, the conidia slightly smaller than *S. haploe*, but again in the same size range as the type description and identical in appearance with the American drawings.

But *S. lepte* in the Old World is consistently more robust in general proportions, and its zygospores are conspicuously larger than the type descriptions, and as such is often indistinguishable from *S. haploe* with proliferated conidiophores.

It is suggestive that here we are dealing with a single variable species, rather than two separate species, but a further note with more conclusive evidence will be published later dealing with English examples of this variable pair. Measurements for the Kenya examples (ranges shown being overall ranges of several samples) are as follows:

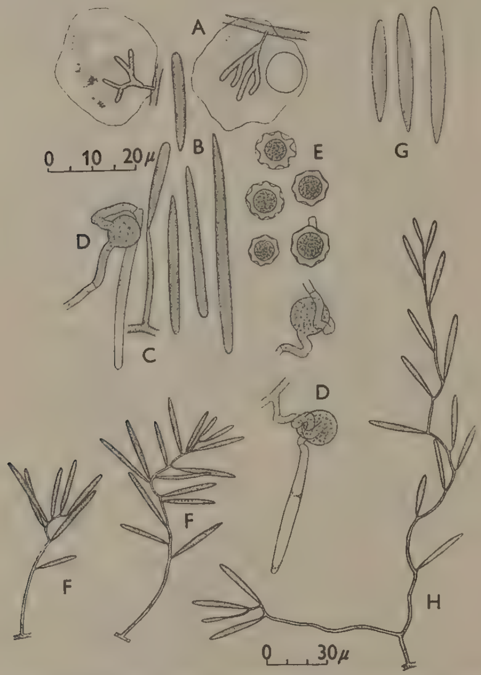

Fig. 10. *Stylopage rhabdospora* Drechsler (A–F, long-spored form; G, H, normal form). A, Haustoria; B, conidia; C, conidiophore; D, formation of zygosporangia; E, zygospores; F (drawn to smaller scale), conidiophores; G, conidia; H, conidiophore (drawn to smaller scale).

S. haploe—most conidia occurring singly. Conidia 10·5–24·5 × 2·3–3·0 µ (Drechsler: 15–25 × 2·3–2·7 µ) and zygospores 6·3–8·2 µ diam. (not recorded by Drechsler). *S. lepte*—all conidiophores bearing 5–7 conidia. Conidia 9·0–21·5 × 2·3–2·7 µ (Drechsler: 12–19 × 1·9–2·7 µ); zygospores 6·0–7·5 µ across (Drechsler: 4·5–6·5 µ).

STYLOPAGE RHABDOIDES Drechsler (Fig. 8)

Only one record of this species was obtained, and the spore size was: 21–36 × 2·5–3·0 µ; i.e. within the *S. rhabdospora* range. Positive identification of this fungus is, however, facilitated by the characteristic conidiophores, which in most cases bear only one conidium, but occasionally up to four when they are borne on long branches, and the zygospores with the conspicuous loose fitting zygosporangium walls.

Apart from the smaller conidia and larger zygospores (7·5–11·6 (protoplast 4·8–7·0) µ diam.), this fungus was in close agreement with the original description (Drechsler, 1947*a*).

Stylopage rhabdospora Drechsler (Fig. 10)

This is a common fungus, both in Kenya and England, and is easily identified by its conidiophore structure and spore size range. The longer-spored form reported by Drechsler appeared on one occasion, with conidia 19·0–49·5 × 2·5–3·0 μ, but otherwise the normal range for this species in Kenya, 24–33 × 2·5–3·3 μ, agreed well with Drechsler (1936). The conidiophores, however, normally carried 7–10 conidia and occasionally more.

Zygospores were produced very readily; 7·2–9·8 (protoplast 4·8–6·7) μ diam.

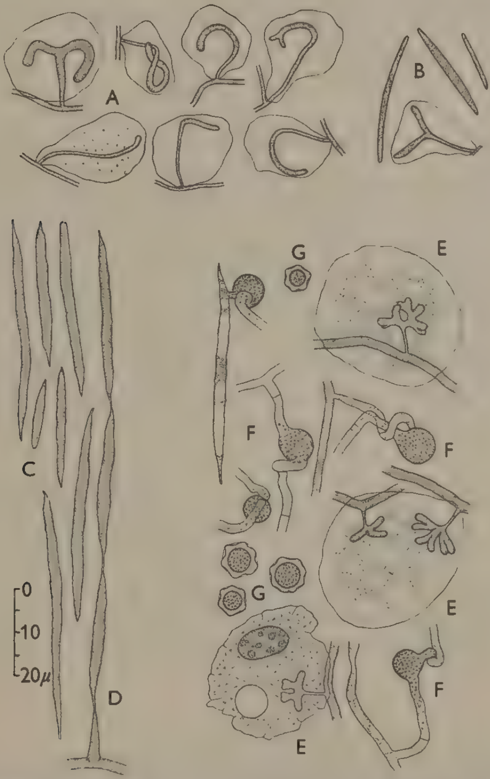

Fig. 11. *Zoopage thamnospira* Drechsler (A, B). A, Haustoria; B, conidia. *Z. atractospora* Drechsler (C–G). C, Conidia; D, conidiophore; E, haustoria; F, zygosporangia; G, zygospores.

Stylopage rhynchospora Drechsler (Fig. 8)

Kenya examples differ only in minor details from the type description (Drechsler, 1939*b*) and in these differences are in close agreement with their English relatives. The fungus is more robust, with the mycelium 2·0–2·8 μ wide, the conidiophores lack the bulbous base, and the conidia are larger—21·7–40·5 × 7·5–12·0 μ (including secondary conidia) and lack the pronounced basal process. Zygospores also are larger, 9·3–15·0 μ diam., excluding the protuberances (12·5–18·0 μ overall) with internal protoplast 7·0–11·5 μ diam.

ZOOPAGE ATRACTOSPORA Drechsler (Fig. 11)

Although differing from Drechsler's (1936) description on several points, there is no doubt of its correct identification. The conidia, borne in chains of 10–13, had irregular, conspicuously warted walls with blunt-pointed ends (except the terminal conidium). The basal conidium sometimes branched to give two conidial chains. Rather larger than those described by Drechsler, the conidia were 21·0–58·5 (mostly 30–45) × 2·2–3·0 μ. Drechsler gives 10–45 × 1·4–2·7 μ. Zygospores were produced readily; 6–9 μ diam. overall (protoplast 3·6–6·0 μ).

Another fungus was obtained and identified as *Z. atractospora*, although its zygospores were considerably larger, 8·0–12·5 μ diam., agreeing in this respect with samples obtained from England.

ZOOPAGE THAMNOSPIRA Drechsler (Fig. 11)

This is probably the commonest Zoopage in England, and certainly the most easily identified from the characteristic haustoria. Only one specimen was obtained from Kenya soils and this differed from the English and American examples in its longer conidia. The conidia were smooth with abruptly rounded tips, 21–48 (mostly 25–38) × 1·5–2·5 μ, borne in chains of 5–8. The rough-walled conidia described in America (Drechsler, 1938) have not been observed elsewhere. The Kenya fungus had mostly simple haustoria, only a small percentage with a single dichotomy; they were also narrower than usual, mostly 1·3–2·0 μ wide at their distal ends. No sexual stages observed.

Type material of *Acaulopage aristata*, *A. retusa*, and *Cochlonema pygmaea* has been deposited in the Herbarium of the Royal Botanic Gardens, Kew.

I wish to express my appreciation to Mr R. J. Jones, who kindly collected most of the soil samples, and also my sincere thanks to Dr C. L. Duddington for his stimulating interest during the course of the work.

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STUDIES ON THE SPECIALIZATION OF *SPHAEROTHECA FULIGINEA* SCHLECHT. (POLL.) AND OTHER POWDERY MILDEWS

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The ability of conidia of *Sphaerotheca fuliginea* from one host to infect other hosts belonging to different genera or families has been demonstrated under Shambat conditions. Conidia from *Cucurbita pepo* or *Cucumis sativus* were able to cause infection of *Hibiscus esculentus* and, similarly, cross-inoculations from *H. esculentus* to *C. pepo* or *C. sativus* were all successful.

With *Leveillula taurica*, it was found that the conidia from *H. esculentus* were able to infect *Faba bona*, *Euphorbia heterophylla* or *Lycopersicon esculentum*. Similarly, inoculations in the reverse direction on to *H. esculentus* were also successful. Furthermore, conidia from *Corchorus olitorius* caused infection of *H. esculentus* but not of *F. bona*.

On the other hand, negative results were obtained with *Erysiphe umbelliferarum* (= ? *E. polygoni*) from *Daucus carota* or *F. bona*. Conidia from either species did not cause an infection on a different host species.

These series of tests using *S. fuliginea* or *L. taurica* yielded positive results when the inoculations were made during the cooler months of December–February. However, when similar inoculations, even between members of the same species, were repeated in March–May, little or no infection occurred in the majority of tests. When there was an infection the incubation period was much longer and the spread was considerably slower. From field observations both mildews are prevalent in the winter, but rare in the summer (April–July). It is believed, therefore, that if there is any appreciable degree of specialization in both species, it is not an inherent character but, like pathogenicity itself, is probably directly affected by the temperature.

INTRODUCTION

Powdery mildews are generally considered to be specialized parasites, and some workers have even demonstrated physiologic races within certain species (Mains & Dietz, 1930; Newton & Cherewick, 1947; Smith & Blair, 1950). On the other hand, other workers have reported tendencies towards a subspecialized habit (Salmon, 1905; Hardison, 1944) or the presence of polyphagous species in *Erysiphe* (Hammarlund, 1945).

In an earlier work at Shambat, the ability of conidia of *Leveillula taurica* (Lév.) Arn. from one host species to infect a wide range of hosts belonging to different families was demonstrated (Nour, 1958). The present work was done under similar conditions and along similar lines. However, in addition to *L. taurica* two other mildew species: *Sphaerotheca fuliginea* Schlecht (Poll.) and *Erysiphe umbelliferarum* de Bary (= ? *E. polygoni* DC.) were tested.

METHODS

The tests were made on the first (cotyledonous) and second leaves of plants in pots. Only half the leaf was inoculated by gently adpressing a diseased leaf containing fresh conidia on to it; the other half was left as control. For each test nine half leaves were inoculated in the early morning and were kept exposed in a sheltered place for 48 hr. before being removed outdoors. Each series of inoculations was repeated once a week during the second half of December 1957 to May 1958. Some series of tests were not done, either due to lack of inoculum or when negative results were consistently obtained.

A leaf was considered infected when the inoculated half showed the characteristic symptoms and produced conidia identical with those of the inoculum, and when the other half (control) was more or less healthy. The conidia thus produced as a result of artificial inoculation were transferred on to healthy leaves of the 'original' host to complete the cycle.

Conidia of *S. fuliginea* were obtained from the field from *Cucurbita pepo* L. (bearing the characteristic cleistothecia) or from *Hibiscus esculentus* L. Conidia from the former species were tested on *H. esculentus*, *Cucumis sativus* L., *Faba bona* Medic., or *C. pepo*, respectively. Similarly, conidia from *H. esculentus* were inoculated on to *C. pepo*, *C. sativus* and *H. esculentus*, respectively. For tests with *L. taurica*, the conidia for inoculation were obtained from *H. esculentus*, *F. bona*, *Euphorbia heterophylla* L., *Corchorus olitorius* L. or *Lycopersicon esculentum* Mill. The inoculum of *E. umbelliferarum* (= ? *E. polygoni*) was obtained from *Daucus carota* L. and *F. bona*.

For all three mildews the host species from which the conidia were obtained are given in the first column and the species inoculated are given in the second column of Table 1. The numerical columns show the total number of half leaves infected over the total of half leaves originally inoculated but still green at the time of sampling. These figures are based on the total of inoculations done during each month. Thus if an inoculation was effected on, say, 25 January, and the final count made on 10 February, then the figures obtained would be included in the January column.

RESULTS

During December to February, the symptoms appeared 7 to 10 days after inoculation, but their appearance was much delayed on the plants inoculated later in March or early April (15–20 days). Although the numerical data in Table 1 are based on the total of the leaves inoculated in the month shown, irrespective of whether the symptoms did appear on those leaves during that or the following month, yet the results clearly indicate that there is a marked progressive decrease in susceptibility with the rise in temperature. It is highest in January or February and lowest or absent in April and May for both *S. fuliginea* and *L. taurica*. Furthermore, both species have shown a remarkable diversity of pathogenicity with definite subspecialized tendencies. *E. umbelliferarum*, on the other hand,

Table 1. *The reaction of five species to inoculation and cross-inoculation using conidia of* Sphaerotheca fuliginea, Leveillula taurica *or* Erysiphe umbelliferarum *as an inoculum*

Species and source of conidia	Test species	Jan.	Feb.	Mar.	Apr.	May
(1) *S. fuliginea*						
C. pepo	*H. esculentus*	27/32	39/47	10/33	0/18	—
	C. sativus	32/35	28/32	9/34	0/32	—
	F. bona	0/35	0/36	—	—	—
	C. pepo	28/28	36/38	20/36	—	—
H. esculentus	*C. pepo*	34/38	27/35	14/77	3/45	0/41
	C. sativus	32/36	30/38	0/35	2/42	0/46
	H. esculentus	36/40	33/42	10/12	4/18	6/29
(2) *L. taurica*						
H. esculentus	*E. heterophylla*	—	—	15/32	4/30	0/13
	F. bona	26/35	7/44	2/46	0/48	—
	L. esculentum	—	—	18/24	1/21	—
F. bona	*H. esculentus*	44/53	15/41	0/24	0/15	—
	C. pepo	0/41	0/29	—	—	—
	F. bona	21/28	6/32	—	—	—
E. heterophylla	*H. esculentus*	25/29	18/27	11/48	0/21	0/16
C. olitorius	*H. esculentus*	15/34	25/38	—	—	—
	F. bona	2/30	3/34	—	—	—
	C. pepo	0/12	0/42	—	—	—
L. esculentum	*H. esculentus*	—	—	25/30	2/26	—
(3) *E. umbelliferarum*						
D. carota	*F. bona*	0/48	0/28	0/35	—	—
	C. pepo	—	0/18	0/12	—	—
	C. sativus	0/20	0/26	—	—	—
	H. esculentus	0/32	0/60	—	—	—
F. bona	*C. pepo*	—	0/22	0/30	—	—
	H. esculentus	—	0/16	0/28	—	—
	F. bona	—	10/16	12/18	—	—
Temperature (° C.)						
Mean max.		30·7	31·3	37·8	42·4	42·5
Mean min.		14·7	14·1	18·6	24·9	26·2

which has more or less identical conidia on both *D. carota* and *F. bona*, gave negative results when the conidia from the former host were deposited on leaves of the latter, even during the month of February when conditions seem to be optimum for its spread.

DISCUSSION

This work has shown that *L. taurica* is even less specialized than has been demonstrated before (Nour, 1958). Likewise, conidia of *S. fuliginea* collected from various hosts are able to infect other host species belonging to different genera and families under winter conditions in the Sudan.

On the other hand, *E. umbelliferarum* has shown strict specialization within the narrow range of the hosts tested in this work. A high proportion of the conidia of this species, whether from *D. carota* or *F. bona*, were more or less cylindrical with almost parallel walls although some barrel-shaped conidia were usually present. The name *E. umbelliferarum* is adopted here (Blumer, 1933) in preference to Salmon's classification where a species having either shape of conidium is named *E. polygoni* (Salmon, 1900).

According to Tarr (1952) the mildew caused by *S. fuliginea* is found on *C. pepo* and *C. sativus* almost wherever they are grown in the Sudan, but is restricted on *H. esculentus* (Okra) to localities south of the Gezira. The Okra grown in and north of the Gezira, according to Tarr, is susceptible to another mildew (*Erysiphe cichoracearum* DC.) which has, therefore, a more northerly distribution. With the apparent absence of cleistothecia on Okra, wherever it is grown in the Sudan, the identification of the pathogen on diseased leaves of this host in the field has so far been roughly based on this geographical zonation of the two pathogens. The distinction between *E. cichoracearum* and *S. fuliginea* based on conidial dimensions alone is still uncertain. The fact that conidia of the latter species, from leaves of *C. pepo* (containing cleistothecia as well), were able to infect Okra at Shambat has, therefore, shown (*a*) that Okra is susceptible to this species well north of the Gezira, and (*b*) that the problem of identification has become somewhat complicated. Unless these two species could be distinguished by vegetative characteristics alone, and with the present knowledge that *S. fuliginea* is not as specialized as has been assumed, it might be likely that *E. cichoracearum* does not even exist in the Sudan, at least on Okra. Conversely, if this species is the pathogen on Okra in Shambat (as according to Tarr) then it is capable also of infecting members of the Cucurbitaceae and it, also, is therefore not strictly specialized.

Whilst specialization was 'weak' for both *L. taurica* and *S. fuliginea* and their pathogenicity 'strong' during the winter, the process of infection became gradually curtailed and specialization more apparent with the rise of temperature during the latter part of March and afterwards. *S. fuliginea* on the two cucurbits was rare or absent in the field after the first week of April, but more prevalent on Okra. When conidia from Okra were placed on leaves of either cucurbit during April no infection was obtained. On the other hand, inoculations from Okra to Okra during the same period gave an infection, though considerably delayed, and the ratio of infected to inoculated leaves was low. It is highly probable, therefore, that the climatic conditions (mainly the high temperature) are the limiting factor to pathogenicity, but it seems likely also that this pathogen becomes more specialized as a result.

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ACAULOPAGE TETRACEROS DRECHSLER VAR. *LONGA* VAR.NOV.

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(With 1 Text-figure)

Since *Acaulopage tetraceros* Drechsler (1935) was first described from America it has been frequently recorded elsewhere; in fact it appears to be the commonest member of the Zoopagales in this country.

Both in England and Kenya, examples of this fungus may be separated into two groups, one closely resembling the type description, the other differing in several details. These two forms can be distinguished mainly on spore shape and size, but the variant shows a preference for a wetter environment and is usually found in ponds and streams where it replaces the type form.

This variant differs from the type in that the sides of the conidium are straighter, with a less abrupt narrowing near the point of attachment, and that the ratio length to breadth of the conidium (excluding basal and distal empty processes) is at least 3·5 and often 5·0 compared to a usual ratio of 2·0 to 2·5 in the type.

The empty distal appendages of the conidium are fewer in number, in most cases 2 or 3, but occasionally 4 or 5. They are variable in length, sometimes only about half the length of the basal living part of the conidium, but often longer and exceeding the living cell.

The size ranges of the conidia, covering the range of fifteen examples from England and Kenya, are as follows: the basal living cell (excluding the empty basal appendage) 23·0–46·5 μ long, 4·8–9·0 μ wide and bearing two to five distal appendages 8–49 μ long. The corresponding dimensions for the type form are 16·5–28·5 $\mu \times$ 6·5–11·5 μ, with two to eight processes (mostly 4) 6·5–22·0 μ long.

Sexual reproduction was not observed.

This fungus has on all occasions appeared distinct from the type form from which it may be readily separated by its spore shape. We consider it advisable to describe this fungus as a variety of *A. tetraceros*, proposing the varietal epithet 'longa' in reference to the shape of the conidia.

Acaulopage tetraceros Drechsler var. **longa** var.nov. (Fig. 1)

Conidia in forma coni inversi, longi 25–41 μ crassi 5·5–9·0 μ, habens 2–4 vacua cornua in apice, longa 18–25 μ, crassa 1·0 μ in pede. Habitat in stagno prope Sidcup, England.

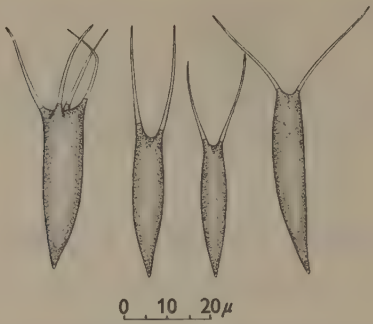

Fig. 1. Conidia of *Acaulopage tetraceros* Drechsler var. *longa* var. nov.

Type material has been deposited in the Herbarium of the Royal Botanic Gardens, Kew.

The authors wish to record their thanks to Mr J. A. Easterling for the Latin diagnosis.

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A COMPARATIVE STUDY OF COMPETITIVE SAPROPHYTIC ABILITY IN TWELVE ROOT-INFECTING FUNGI BY AN AGAR PLATE METHOD

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The agar plate method is a modification of the 'Cambridge method' for the estimation of competitive saprophytic ability, as developed by F. C. Butler and R. L. Lucas. A pure culture of the fungus to be tested is grown upon maizemeal-sand medium and mixed with an unsterilized soil in a series of dilutions. From these inoculum-soil mixtures, solidified by impregnation with water agar, disks are cut out and plated upon Czapek-Dox + 0·05% yeast-extract agar, acidified to pH 4·0.

By this method, the following were classed as vigorous saprophytes: *Fusarium culmorum*, *F. avenaceum*, *F. oxysporum* f. *cubense*, *F. oxysporum* f. *vasinfectum*, *F. caeruleum* and *Curvularia ramosa*. Saprophytes of intermediate ability were *Helminthosporium sativum* (isolate 1) and *Macrophomina phaseoli*. The following behaved as poor saprophytes: *Verticillium dahliae*, *H. sativum* (isolate 2), *Rhizoctonia solani*, *Fomes annosus* and *Ophiobolus graminis*. These results agree well enough with earlier observations by other methods, except for the poor saprophytic performance on the agar plate of *M. phaseoli* and *R. solani*, which can certainly colonize, as competitive saprophytes, certain substrates in the soil. It seems, therefore, that the agar plate method provides conditions of competition more intense than those associated with some natural substrates in the soil.

The term 'competitive saprophytic ability' was introduced by Garrett (1950), in discussing the observed failure of some specialized root-infecting fungi to compete successfully with less specialized parasites and with obligate saprophytes for colonization of dead organic material in the soil. Garrett suggested that a high degree of competitive saprophytic ability was incompatible with continued evolution of the host-parasite relationship, and concluded that such evolution was likely to be accompanied by a progressive loss of competitive saprophytic ability. Further support for this general proposition was later provided by the experimental work of Butler (1953*a–c*) and Lucas (1955), who developed the 'Cambridge method' for estimating competitive saprophytic ability. Other methods have been devised by Park (1958, 1959) in an independent approach to the same general problem. More recently, Garrett (1956, p. 130) has defined competitive saprophytic ability as 'the summation of physiological characteristics that make for success in competitive colonization of dead organic substrates'.

The present paper reports an attempt to provide a rapid method for comparing competitive saprophytic ability in a number of root-infecting fungi. In discussing competitive saprophytic colonization, Garrett (1956, ch. 7) has pointed out that the isolation plate, as used by plant pathologists for the isolation of fungal parasites from pieces of surface-sterilized, infected host tissue, serves as a useful model of saprophytic competition for substrates in the soil. The fragment of surface-sterilized tissue thus plated-out on nutrient agar contains, more often than not, secondary parasites and even obligate saprophytes that have followed the primary parasite into the disorganized host tissues. Competition for colonization of the nutrient agar from this mixed inoculum therefore follows the plating-out of the plant tissue fragment. The most frequent fungal colonist in such platings is not the primary parasite, if it is a specialized parasite of low competitive saprophytic ability, but a following secondary parasite or obligate saprophyte.

The use of this agar plate model of saprophytic competition as an illustration by Garrett suggested that it might be put to more practical and systematic use for the investigation of competitive saprophytic ability. The 'Cambridge method', as originally developed, was therefore modified for use with agar plates, as described below. It must be admitted that the agar plate, as a model, provides only a partial analogy with the more complex situation in the soil. Secondly, the standardization of procedure that has been necessary for speed and economy of effort in the comparison of a sufficient number of fungi has inevitably vitiated the validity of the comparisons, inasmuch as conditions that are optimum for one fungus cannot be optimum for all. The results obtained by this method are therefore presented below as a preliminary to, rather than as a substitute for, more intensive autecological studies of the kind carried out by Park (1958, 1959) with the form of *Fusarium oxysporum* causing vascular wilt of the oil palm.

MATERIALS AND METHODS

The agar plate method

By the 'Cambridge method', pure cultures on maizemeal-sand medium of the fungi to be tested are diluted with increasing proportions of a moist, unsterilized soil. In this series of inoculum-soil mixtures are incubated, for 1 month at laboratory temperature, segments of autoclaved tissue of the appropriate host plant. At the end of the incubation period, 100 colonized segments from each inoculum-soil mixture are tested for colonization by the inoculant fungus. Butler and Lucas both worked with fungi causing root and foot rots of cereals, and so segments of autoclaved wheat straw were used as the test substrate. Two alternative tests were chiefly employed to detect colonization of segments by the inoculant fungus: (1) Garrett's (1938) wheat seedling test, (2) a 'sand plate method' devised by Butler (1953*a*) for sporulating fungi, whereby the segments of wheat straw were incubated on moist sterilized sand in Petri dishes, to promote sporulation. The 'Cambridge method' thus tests the ability of a particular fungus to colonize a selected substrate in competition with the microflora of a natural soil, in a dilution series in which the inoculum potential of the inoculant fungus is progressively decreased in relation to that of the natural soil microflora. With *any* inoculant fungus, the percentage of substrate units that are colonized decreases progressively with decrease in inoculum potential of the inoculant fungus down the dilution series. But the *rate* at which percentage colonization of substrate units decreases down the dilution series is an inverse function of the competitive saprophytic ability of the inoculant fungus; thus curves of shallow slope are given by fungi of high competitive saprophytic ability, and curves of steep slope by fungi of low competitive saprophytic ability.

In the agar plate modification a dilution series of inoculum-soil mixtures is prepared as before. Each inoculum-soil mixture is spread over the bottom of a sterile Petri dish, and over it is then poured a layer of sterile distilled-water agar, cooled to 40° C., so as to impregnate the layer of soil. When the agar has set, disks of the inoculum-soil mixture held together by agar are cut out with a sterilized cork borer and four disks from each mixture are placed equidistantly on each of five plates of acidified Czapek-Dox + yeast extract agar. After a suitable incubation period, the number of colonies formed by the inoculant fungus, from a total of twenty disks of each inoculum-soil mixture that were plated-out, is recorded.

Preparation of inoculum. Maizemeal-sand medium (in proportions of 3 g. maizemeal: 100 g. dry sand: 15 ml. distilled water) was autoclaved in lots of 170 g. in 250 ml. conical flasks for 1 hr. at 22 lb. After inoculation with three 6 mm. disks taken from the growing margin of an agar colony, flasks were incubated for a standard period of 28 days at 25° C.; this period was adopted in the original 'Cambridge method' in order to reduce unused nutrients in the maizemeal-sand cultures to a minimum.

Selection and preparation of soils. The soils used in parallel series in these experiments were: (1) Kettering soil—a medium loam with a fairly high organic and total nitrogen content, collected from under grass; pH 5·0–6·0; moisture-holding capacity (M.H.C.) 65–70 ml./100 g. air-dry soil (by perforated box method). (2) Cambridge Botanic Garden soil—a light loam; pH *c.* 7·2; M.H.C. 50 ml./100 g. air-dry soil. Samples of soil for an experiment were collected from the field (from a field dump in the case of Kettering soil) and adjusted to a moisture content of 40 % M.H.C. before use.

Preparation of inoculum-soil mixtures. The six mixtures selected as a standard range for all experiments contained the following percentages (by weight) of maizemeal-sand inoculum: 98, 90, 75, 50, 25 and 10. Inoculum and soil for each dilution were mixed thoroughly by hand on a clean fresh sheet of glazed brown paper; 10 g. of each mixture was then spread out as an even layer in the bottom of a sterile Petri dish, and over this was poured 10 ml. sterile distilled-water agar, cooled to 40° C. After the agar had set, disks were cut out from the agar impregnated inoculum-soil mixtures with a sterilized no. 1 size cork borer (4 mm. diam.) in preparation for inoculation of the Czapek-Dox agar plates.

Inoculation of Czapek-Dox agar plates. A slightly modified Czapek-Dox + yeast extract agar was employed: $NaNO_3$, 2 g.; KH_2PO_4, 1 g.; KCl, 1 g.; $MgSO_4 . 7H_2O$, 0·5 g.; $FeSO_4$, 0·01 g.; 'Bacto' yeast extract, 0·5 g.; sucrose, 30 g.; agar, 22·5 g.; distilled water, 1000 ml. The medium was acidified to pH 4·0 with phosphoric acid and autoclaved for 10 min. at 10 lb.; 20 ml. medium was allocated to each 9 cm. Petri dish. The medium was thus similar to that chosen by Warcup (1950) for his 'soil plate method'; Warcup (1959) states that his medium contains 0·05 % yeast extract, and that 0·5 % was given in error. After setting, plates were inverted and left for 24 hr. before use, to permit evaporation of the surface film of moisture, which is known to encourage excessive bacterial development even on acidified agar. Five plates were then inoculated equidistantly with four inoculum-soil disks each, so as to give twenty disks from each inoculum-soil mixture.

Assessment and recording of results. Recognizable colonies of the inoculant fungus were recorded for each plate after 10 days. Mixed fungal colonies developed from some of the inoculum disks, and so the total fungal growth was divided into four sectors. For each inoculum disk, the inoculant fungus was assigned a rating of 1, $\frac{3}{4}$, $\frac{1}{2}$, $\frac{1}{4}$ or 0, according to the proportion of the total fungal colony that it occupied. The ratings of the inoculant fungus for each of the twenty inoculum disks were then added together and the total was multiplied by five, to give a percentage figure.

Fungi selected for study

The twelve root-infecting fungi selected for study are listed below, together with the species of host plant from which each one was isolated, and the names of those mycologists who kindly supplied the original cultures:

	Isolated from	Supplied by
Curvularia ramosa (Bain.) Boedijn	Wheat	Dr F. C. Butler
Fomes annosus (Fr.) Cooke	Pine	Dr J. Rishbeth
Fusarium avenaceum (Fr.) Sacc.	Potato	Dr R. K. McKee
F. caeruleum (Lib.) Sacc.	Potato	Dr M. J. Carlile
F. culmorum (W. G. Sm.) Sacc.	Wheat	Dr S. D. Garrett
F. oxysporum Fr. f. *cubense* (E. F. Smith) Snyder & Hansen	Banana	Dr J. Rishbeth
F. oxysporum f. *vasinfectum* (Atk.) Snyder & Hansen	Cotton	Prof. T. S. Sadasivan
Helminthosporium sativum, Pammel, King & Bakke (isolates 1 and 2)	Wheat	Dr F. C. Butler
Macrophomina phaseoli (Maubl.) Ashby	Cotton	Prof. T. S. Sadasivan
Ophiobolus graminis (Sacc.) Sacc.	Wheat	Dr S. D. Garrett
Rhizoctonia solani Kühn	Swede	Dr R. K. S. Wood
ticillium dahliae Kleb.	Cotton	Dr G. M. Wickens

Two isolates of *Helminthosporium sativum* were employed in these experiments; isolate 1 was a recent isolate supplied by Dr F. C. Butler, and showed a staling habit of growth on agar plates. Isolate 2 was the original isolate employed by Butler (1953*a–c*).

EXPERIMENTAL RESULTS FROM THE AGAR PLATE METHOD

The twelve fungi chosen for investigation were tested in three groups of four, since 120 plates was about the maximum that could conveniently be handled in one trial (i.e. 4 fungi × 6 inoculum-soil mixtures × 5 = 120 plates). Accordingly, a test was made with each group using Kettering soil in the inoculum-soil mixtures and then a parallel test was set up with Botanic Garden soil. The first group of species was chosen to permit an exact comparison with the results obtained by Butler (1953*a*) for the same four species by the 'Cambridge method'; results for Kettering soil are given in Table 1 and for Botanic Garden soil in Table 2.

Results compare reasonably well with those obtained by Butler; thus both *Fusarium culmorum* and *Curvularia ramosa* were as vigorous in colonization of the nutrient agar as they had been in colonization of Butler's wheat straw. *Helminthosporium sativum* again proved to be a poorer competitive saprophyte than either of the foregoing species, and *Ophiobolus graminis* did not produce a single recognizable colony in either soil series. In respect of *O. graminis*, therefore, the agar plate method has given a wider separation than the original 'Cambridge method'.

Table 1. *Saprophytic colonization of agar plates against Kettering soil microflora*

(Percentage colonization.)

% inoculum	*O. graminis*	*F. culmorum*	*C. ramosa*	*H. sativum* (1)	*H. sativum* (2)
98	0	100	93	66	34
90	0	98	84	53	19
75	0	95	78	33	11
50	0	93	69	23	5
25	0	91	55	18	3
10	0	89	33	13	0
Mean	0	94	69	34	12

Table 2. *Saprophytic colonization of agar plates against Botanic Garden soil microflora*

(Percentage colonization.)

% inoculum	*O. graminis*	*F. culmorum*	*C. ramosa*	*H. sativum* (1)	*H. sativum* (2)
98	0	100	98	94	83
90	0	98	86	78	55
75	0	96	80	75	39
50	0	91	76	58	15
25	0	89	68	45	8
10	0	76	50	23	3
Mean	0	92	76	62	34

The second group to be tested contained the remaining four Fusaria. For this group, two modifications of the standard method had to be made. First, pure cultures were incubated at 22·5° C. instead of at 25° C., which latter temperature is too high for *F. caeruleum*. Secondly, the standard Czapek-Dox + yeast-extract agar proved to be unsuitable, because *F. caeruleum*, *F. oxysporum cubense* and *F. oxysporum vasinfectum* failed to produce sufficient pigment for easy identification of colonies on this medium. Brown (1925) had originally shown for *F. fructigenum*, and Carlile (1956) later demonstrated for *F. oxysporum*, that pigment production was favoured by a high carbon-nitrogen ratio in the medium. A preliminary trial showed that Czapek-Dox + yeast-extract agar with $\frac{1}{4}$ standard concentration of nitrogen (i.e. 0·5 g. instead of 2·0 g. $NaNO_3$/l.) permitted good pigment production without much reduction in the density of mycelial growth, and so this modified agar was employed for these four Fusaria (Tables 3 and 4).

Table 3. *Saprophytic colonization of agar plates against Kettering soil microflora*

(Percentage colonization.)

% inoculum	*F. avenaceum*	*F. caeruleum*	*F. o. cubense*	*F. o. vasinfectum*
98	100	95	100	100
90	95	86	98	99
75	84	79	94	95
50	76	65	85	83
25	69	54	64	59
10	36	53	21	19
Mean	77	72	77	76

Table 4. *Saprophytic colonization of agar plates against Botanic Garden soil microflora*

(Percentage colonization.)

% inoculum	*F. avenaceum*	*F. caeruleum*	*F. o. cubense*	*F. o. vasinfectum*
98	99	99	100	99
90	95	88	96	95
75	89	75	86	89
50	78	65	75	79
25	63	44	50	49
10	43	25	33	31
Mean	78	66	73	74

Although none of these Fusaria was as vigorous as *F. culmorum*, yet all four can be grouped together with *F. culmorum* and *Curvularia ramosa* as vigorous competitive saprophytes on the agar plate.

The remaining four species were tested in the third group. For one species only, *Verticillium dahliae*, a modified medium was used; for this species, the Czapek-Dox + yeast-extract agar was prepared at 1/10 standard strength, and on this diluted medium *V. dahliae* regularly produced microsclerotia and verticillate conidiophores permitting easy identification. On full strength medium, on the other hand, *V. dahliae* tended to produce hyaline colonies (Isaac, 1946; Kamal & Wood, 1956), which were difficult to recognize in mixed culture. Apart from this modification for *V. dahliae*, the experiments were set up according to the standard procedure (Tables 5 and 6).

Tables 5 and 6 show that *Fomes annosus* behaved like *Ophiobolus graminis*

Table 5. *Saprophytic colonization of agar plates against Kettering soil microflora*

(Percentage colonization.)

% inoculum	*Fomes annosus*	*M. phaseoli*	*R. solani*	*V. dahliae*
98	0	63	21	53
90	0	43	10	46
75	0	25	4	33
50	0	5	0	18
25	0	3	0	6
10	0	0	0	3
Mean	0	23	6	27

Table 6. *Saprophytic colonization of agar plates against Botanic Garden soil microflora*

(Percentage colonization.)

% inoculum	*Fomes annosus*	*M. phaseoli*	*R. solani*	*V. dahliae*
98	0	99	56	45
90	0	71	26	38
75	0	50	10	30
50	0	19	4	13
25	0	11	0	8
10	0	6	0	3
Mean	0	43	16	23

(Tables 1 and 2), as it failed to produce a single recognizable colony in either soil series. This result was not unexpected, inasmuch as Rishbeth's (1950, 1951 *a*, *b*) extensive observations suggest that *F. annosus* is a specialized root-infecting fungus. The poor saprophytic performance of *Verticillium dahliae* can be contrasted with the vigorous saprophytic behaviour of the other two vascular parasites, *Fusarium oxysporum cubense* and *F. o. vasinfectum* (Tables 3 and 4). *Macrophomina phaseoli* and *Rhizoctonia solani* are the only species to have behaved in an unexpected manner in these tests, inasmuch as their performance on the agar plate is difficult to reconcile with evidence for their saprophytic activity in the soil. Possible reasons for this discrepancy will be considered in the Discussion.

FACTORS AFFECTING RESULTS FROM THE AGAR PLATE METHOD

Incubation period of pure cultures on maizemeal-sand medium

One possible factor affecting validity of results obtained both by the 'Cambridge method', and by this agar plate modification, is variation in vigour of the pure culture inoculum grown on maizemeal-sand medium. This possibility was recognized, though not experimentally tested, by Lucas (1955), in discussing fluctuations in the general level of saprophytic colonization of wheat straw by *Ophiobolus graminis* from one experiment to another. Lucas suggested that the peak of vegetative vigour might be attained in different batches of maizemeal-sand cultures after different periods of incubation, and that consequently the vigour of pure cultures at the time of setting-up experiments might vary inversely with the time between peak vigour and end of the incubation period. Ideally, each fungus should be tested at peak vigour of its inoculum; in actual practice, this requirement has proved difficult to reconcile with the plan for an extensive investigation in a limited period. Nevertheless, as a compromise, the effect of incubation period of the inoculum has been investigated with four fungi, so as to evaluate the probable size of error introduced by adoption of a standard incubation period of 28 days at 25° C. Accordingly, cultures of these four fungi were tested against Kettering soil after incubation periods of 14, 21, 35 and 42 days, respectively (Tables 7–10). A 28-day incubation period was not included in this experiment, but figures for this period have been taken from Tables 1–6 and included in Tables 7–10 for ease of comparison.

Table 7. *Saprophytic colonization of agar plates by* Curvularia ramosa *against Kettering soil microflora*

(Percentage colonization.)

% inoculum	Incubation period of pure cultures (days) 14	21	28	35	42
98	93	90	93	85	61
90	85	79	84	69	38
75	80	73	78	60	25
50	76	66	69	45	15
25	63	56	55	31	10
10	48	38	33	21	8
Mean	76	71	73	58	33

Table 8. *Saprophytic colonization of agar plates by* Helminthosporium sativum (1) *against Kettering soil microflora*

(Percentage colonization.)

% inoculum	Incubation period of pure cultures (days) 14	21	28	35	42
98	70	85	66	64	66
90	55	69	53	45	33
75	45	65	33	30	15
50	36	55	23	35	13
25	20	23	18	23	9
10	10	14	13	14	4
Mean	39	52	34	35	23

Table 9. *Saprophytic colonization of agar plates by* Helminthosporium sativum (2) *against Kettering soil microflora*

(Percentage colonization.)

% inoculum	Incubation period of pure cultures (days) 14	21	28	35	42
98	76	70	34	38	18
90	55	45	19	20	8
75	43	34	11	11	6
50	23	16	5	5	3
25	14	9	3	1	3
10	9	3	0	0	0
Mean	37	30	12	13	6

Table 10. *Saprophytic colonization of agar plates by* Rhizoctonia solani *against Kettering soil microflora*

(Percentage colonization.)

% inoculum	Incubation period of pure cultures (days) 14	21	28	35	42
98	35	34	21	23	34
90	13	20	10	11	15
75	6	3	4	5	6
50	0	3	0	0	3
25	0	0	0	0	0
10	0	0	0	0	0
Mean	9	10	6	7	10

Peak vigour of the inoculum was attained by *Curvularia ramosa* and by *Helminthosporium sativum*, isolate 2, after 14 days, and by *H. sativum*, isolate 1, after 21 days. Although colonization figures for *Rhizoctonia solani* fluctuated over the incubation period of 14–42 days, there was no obvious peak period of vigour. Tables 7 and 10 show that the adoption of a standard incubation period of 28 days has not appreciably diminished the level of saprophytic colonization by *C. ramosa* and by *R. solani* below that attained with inoculum at peak vigour. A clearly significant reduction in colonization has resulted from the use of 28-day inoculum in *H. sativum*, isolate 1, and a still greater reduction with isolate 2. Nevertheless, the error is not large enough to affect the broad general conclusion (see Discussion) that the competitive saprophytic ability of *H. sativum* is intermediate between that of the really vigorous saprophytes (e.g. the Fusaria) and that of the poor saprophytes (e.g. *Ophiobolus graminis* and *Fomes annosus*).

Intensity of sporulation on maizemeal-sand medium

In his discussion of possible factors affecting saprophytic colonization of a substrate by a particular micro-organism, Garrett (1956, ch. 7) has listed the following: (1) competitive saprophytic ability of the particular micro-organism, (2) inoculum potential of the micro-organism, (3) environmental factors, including the population of competing micro-organisms. The 'Cambridge method' is based upon the use of a range of inoculum potentials in the trial of each inoculant fungus. For any one fungus, the inoculum potential is directly related to the weight of maizemeal-sand inoculum in the inoculum-soil mixture, but this may not provide an absolute basis for comparison of competitive saprophytic ability between different fungi. Thus inoculum potential for saprophytic colonization has been defined by Garrett (1956, p. 41) as 'the energy of growth of a fungus (or other micro-organism) available for colonization of a substrate at the surface of the substrate to be colonized'. In another context (inoculum potential for root infection), Garrett (1956, p. 196) has related 'energy of growth' to the following combination: (1) the number of units or propagules of the fungus per unit area of surface to be colonized, (2) the nutritional status of such propagules. For a fungus, therefore, inoculum potential is probably a function of the number and growth vigour of active hyphal tips per unit weight of inoculum. It seems possible, therefore, that equal weights of inoculum may not provide equivalent inoculum potentials for the comparison of competitive saprophytic ability in different species of fungi (or even for different strains within a species), though it is alternatively possible to interpret Garrett's definition of competitive saprophytic ability as including within it such interspecific differences in inoculum potential per unit weight of inoculum.

A mere recognition of this problem of definitions must suffice for the present, and we can now consider the more immediate problem as to the proportion of inoculum potential that is contributed by germinating spores. If this proportion is appreciable, then we might expect to find some correlation between percentage saprophytic colonization in these experiments and the intensity of sporulation by different species of fungi on the maizemeal-sand inoculum. Preliminary inspection of the results given in Tables 1–6 suggested that such a correlation might be found, and so spore counts were made on two series of maizemeal-sand inocula.

The first series of spore counts was made on the maizemeal-sand inocula prepared for the experiments on incubation period of the inoculum, described above. No spore counts needed to be made on cultures of the sterile mycelium of *Rhizoctonia solani*, and it was further found that the mycelium of *Helminthosporium sativum*, isolate 2, had become similarly sterile, so that spore counts could be made only on the cultures of *Curvularia ramosa* and *H. sativum*, isolate 1. At the end of the incubation period for each batch of cultures, spore suspensions of each fungus were prepared by agitating 10 g. culture in 100 ml. distilled water and filtering through fine muslin. A droplet of spore suspension was spread over a haemocytometer slide, and the spore density was estimated from the mean of five counts for each suspension. For the purpose of these determinations, one flask inoculated with each fungus had been incubated for 28 days, though the 28-day period had not been included in the experiments on effect of inoculum age (Table 11).

Table 11. *Spore density in maizemeal-sand cultures*

(No. spores/g. culture medium (thousands).)

	Incubation period of pure cultures (days)				
	14	21	28	35	42
C. ramosa	1882	3222	4040	4220	5504
H. sativum (1)	660	876	1224	1200	1250

At first sight, a comparison of Table 11 with Tables 7–10 suggests that spore density of the inoculum can be correlated with percentage saprophytic colonization, both of which are highest for *Curvularia ramosa* and lowest for the two sterile fungi. But if we attempt to correlate spore density with percentage colonization within a single table, i.e. for either *C. ramosa* or *Helminthosporium sativum* at different ages of the inoculum, no simple correlation can be found. Thus peak vigour of the inoculum for saprophytic colonization was attained by *C. ramosa* after 14 days, and by *H. sativum* after 21 days. Yet spore density of the inoculum went on increasing for *C. ramosa* up to 42 days, and for *H. sativum* up to 28 days.

The tentative conclusion that there was no simple correlation between spore density of the inoculum and percentage saprophytic colonization was further tested by setting-up additional maizemeal-sand cultures of *Fusarium culmorum*, *F. avenaceum*, *F. caeruleum*, *F. oxysporum cubense*, *F. o. vasinfectum*, *Fomes annosus* and *Verticillium dahliae*, which were incubated for the standard period of 28 days. Numbers of spores/g. culture were then determined as described above, and the counts are given in Table 12, together with 'saprophytic colonization ratings' for comparison; these ratings have been calculated by averaging percentage colonization figures over the range of six inoculum-soil mixtures for both Kettering and Botanic Garden soils, from the data given in Tables 1–6.

Inspection of the figures given in Table 12 fails to reveal any simple correlation between spore count and saprophytic colonization rating.

Table 12. *Spore density in maizemeal-sand cultures after 28 days compared with mean percentage saprophytic colonization*

	F. culmorum	*F. avenaceum*	*F. o. cubense*	*F. o. vasinfectum*	*F. caeruleum*	*V. dahliae*	*Fomes annosus*
Colonization rating	93	78	75	75	69	25	0
No. spores/g. medium	11,680	616	31,520	4672	2784	38,560	0

Test of saprophytic colonization by non-sporing mycelia

As a further check upon the reliability of results obtained by the standard agar plate modification of the 'Cambridge method', and as an additional precaution against the possibility that results might be biased by the degree of sporulation by different fungi on maizemeal-sand medium, a method was devised whereby colonization of agar plates *by non-sporing mycelia* might be compared for all the fungi already investigated. Non-sporing mycelia on agar were obtained by cutting out 4 mm. disks from the extreme margins of 5-day colonies on Czapek-Dox + yeast extract agar. A simple precaution, described by Brown (1923), was taken to prevent heavy marginal staling and sporulation by *Helminthosporium sativum*, isolate 1. Following Brown's technique, older portions towards the centre of colonies were removed every day with a sterile scalpel, thus preventing the outwards diffusion of staling products and leaving freely growing colony margins with little spore production. Before inoculum disks were cut out, Kettering soil, finely sieved and with moisture content at 45 % M.H.C., was spread over the plates and then excess soil was tipped off. From these soil-dusted plates, twenty 4 mm. marginal inoculum disks were cut out for the inoculation of five acidified (pH 4·0) Czapek-Dox + yeast-extract agar plates with each fungus. Assessment of percentage saprophytic colonization by each of the thirteen fungi was made in the usual way (Table 13).

Table 13. *A comparison of saprophytic colonization from soil-dusted agar inoculum disks with that from a mixture of 98 % maizemeal-sand-inoculum + 2 % soil*

(Percentage colonization.)

	From agar inoculum	From inoculum-soil mixture*
F. culmorum	100	100
F. avenaceum	100	100
F. o. cubense	100	100
F. o. vasinfectum	99	100
F. caeruleum	93	95
C. ramosa	86	93
H. sativum (1)	85	66
H. sativum (2)	84	34
V. dahliae	56	53
M. phaseoli	55	63
R. solani	35	21
Fomes annosus	5	0
O. graminis	3	0

* Figures for comparison taken from Tables 1, 3 and 5.

Table 13 shows that by the soil-dusted mycelial agar disk method the thirteen fungi are placed in the same order (with the single exception of *H. sativum*, isolate 2, having changed places with *Macrophomina phaseoli*) as by the standard agar plate method.

Discussion

In order to contain this investigation within practicable limits, it has been necessary to adopt a standardized procedure, for comparison of competitive saprophytic ability, and this has been followed throughout, with only minor departures essential for recognition of certain individual fungi. Several criticisms can be made of this procedure. First, although a nutrient agar plate is not so very different from the natural saprophytic substrates of some fungi (e.g. the saprophytic sugar fungi), for other fungi it may be much less like the natural substrate, and so colonization of the agar plate will not necessarily reflect colonization of natural substrates in the soil. Secondly, all experiments were carried out at 22·5° C.; this temperature is considerably higher than the mean soil temperature in temperate regions, and it is probable that different results would have been obtained if a lower temperature of incubation had been employed. Thirdly, a standard incubation period for the pure cultures on maizemeal-sand medium was employed throughout the investigation for cultures of all the fungi tested; as shown in Tables 7–10, this has given some advantage to those fungi in which the standard period of incubation has come nearest to the optimum period for peak vigour of the pure cultures. To set against these various disadvantages, the agar plate method as developed and used here has the one advantage of speed and simplicity, and this has enabled a wide comparative survey of a number of fungi to be made in a limited period. Nevertheless, the faults listed above are not radically inherent in the technique, which could be modified to test other substrates presented in the same way; other temperatures of incubation could be tested; all fungi,could be tested at peak vigour of the maizemeal-sand inoculum by using a range of incubation periods, as was done for only four fungi in the present investigation (Tables 7–10).

The data on saprophytic colonization obtained by the agar plate method are given in Tables 1–6 and summarized in Table 14. A 'saprophytic colonization rating' for each fungus has been obtained by calculating the mean percentage colonization for the whole range of inoculum-soil mixtures, from 98 % down to 10 % inoculum. Table 14 shows for each fungus the rating obtained with each of the two soils used in making up the inoculum-soil mixtures, and also a final rating derived from the mean of these two individual soil ratings.

The thirteen fungi (including two forms of *Fusarium oxysporum* producing vascular wilts and two isolates of *Helminthosporium sativum* treated separately) as listed in Table 14 have been divided into three groups, on the basis of their saprophytic colonization ratings. Although there are several anomalies, yet this tentative grouping agrees reasonably well with such evidence on saprophytic behaviour as is available from other sources. Thus earlier workers have concluded that both *Fusarium culmorum* (Sadasivan, 1939; Walker, 1941; Butler, 1953*a*; Lucas, 1955) and *Curvularia ramosa* (Butler, 1953*a*, *b*) are vigorous competitive saprophytes. Two other species of *Fusarium* and two wilt-producing forms of *F. oxysporum* are also included in group I, as vigorous saprophytes on the agar plate. Garrett (1956, p. 66) has concluded that the wilt-producing forms of *F. oxysporum* are probably soil-inhabiting saprophytes; more recently Park (1958, 1959) has produced convincing experimental evidence of a new kind for the same conclusion with respect to the form of *F. oxysporum* causing vascular wilt of the oil palm.

Table 14. '*Saprophytic colonization ratings*' *summarized from Tables 1–6*

		From inoculum-soil mixtures with		
		Kettering soil	Botanic Garden soil	Mean
Group I, vigorous saprophytes	*F. culmorum*	94	92	93
	F. avenaceum	77	78	78
	F. o. cubense	77	73	75
	F. o. vasinfectum	76	74	75
	C. ramosa	69	76	73
	F. caeruleum	72	66	69
Group II, intermediate	*H. sativum* (1)	34	62	48
	M. phaseoli	23	43	33
Group III, poor saprophytes	*V. dahliae*	27	23	25
	H. sativum (2)	12	34	23
	R. solani	6	16	11
	Fomes annosus	0	0	0
	O. graminis	0	0	0

Turning next to the poor saprophytes listed in Group III, neither *Ophiobolus graminis* nor *Fomes annosus* produced a single recognizable colony throughout the investigation with the standard agar plate method, though a few colonies of each fungus were produced by another method (soil-dusted mycelial agar disks: Table 13). This result agrees with expectation from earlier evidence for *O. graminis* and is not at variance with a wide variety of observations on *F. annosus* made by Rishbeth (1950, 1951*a*, *b*). The grouping of *Helminthosporium sativum*, isolate 2, as a poor saprophyte agrees with the conclusion of Butler (1953*a*, *b*) working with the same isolate; the behaviour of this species may be somewhat variable, however, as a more recent isolate (1) has been placed in group II of intermediate saprophytes. No previous evidence about the saprophytic behaviour of *Verticillium dahliae* is available, but in these experiments on the agar plate it has behaved as a poor saprophyte.

Variation in the saprophytic behaviour of the two isolates of *H. sativum* has already been noted. Two other fungi have shown unexpected behaviour on these agar plates. Thus *Macrophomina phaseoli* is classed in group II as having only intermediate saprophytic ability, yet Zachariah (1953) has shown it to be a vigorous competitive colonizer of autoclaved cotton root segments buried in the soil, and Kovoor (1954) reported extensive growth of its mycelium through unsterilized soil, for distances too great for the mycelium to have been nourished solely by the nutrient reserves of the inoculum. A similar observation had earlier been made by Blair (1943) for *Rhizoctonia solani*, yet he also demonstrated that this fungus is sensitive to the competition of other soil micro-organisms, inasmuch as its saprophytic growth was retarded or even inhibited by the addition of fresh organic material to the soil. *R. solani*, in so far as its saprophytic growth through soil is concerned, could thus be classed in Winogradsky's (1925) group of autochthonous soil micro-organisms, active only in soil that has not recently been supplied with fresh organic material. *R. solani* falls into Group III of poor saprophytes on the basis of its performance in these agar plate tests.

We can only conclude, therefore, that the agar plate method, as used here, has provided conditions of competition more severe than those that occur on some natural substrates in the soil. It seems likely that some fungi, such as *M. phaseoli* and *R. solani*, which are poor or only moderately successful competitive saprophytes on these nutrient agar plates, are yet successful saprophytes under other conditions—perhaps of less intense competition—in the soil. In respect of such fungi, therefore, the agar plate method will prove an unreliable guide to saprophytic behaviour in the soil.

I wish to express my gratitude to Dr S. D. Garrett, who suggested the problem and supervised the work, for his encouragement and help.

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POLYCLADIUM EQUISETI GEN.NOV., SP.NOV.: AN AQUATIC HYPHOMYCETE ON *EQUISETUM FLUVIATILE*

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(With 1 Text-figure)

Polycladium equiseti gen.nov., sp.nov., a submerged aquatic hyphomycete found on dead internodes of *Equisetum fluviatile*, is described. The simple conidiophore grows by means of a persistent apical cell. Conidia are sessile and lateral. Each consists of a long filamentous main axis bearing two to four lateral branches one or two of which may branch again. The new genus is compared with *Tricladium* and *Varicosporium*.

The species described in this paper was found in April 1958 growing on submerged, disarticulated, bleached internodes of *Equisetum fluviatile* lying in still water at a depth of 10–30 cm. near the outflow end of Blea Tarn in Westmorland. About a hundred of these internodes were examined and the fungus was seen on approximately one in eight. Two other aquatic Hyphomycetes (*Varicosporium elodeae* and *Articulospora tetracladia*) were present with about the same frequency of occurrence. Further, nearly all the internodes carried numerous perithecia of *Loramyces macrospora*. All these fungi were to be seen on *Equisetum* internodes taken directly from the Tarn, no incubation in the laboratory being necessary for their development.

The mycelium occurs within the substratum and the conidiophores alone project into the water (Fig. 1). The conidiophore is rather long, unbranched and septate, the cross-walls being conspicuous. It increases in length by a rather pointed apical cell with dense protoplasmic contents. The spores are borne laterally on the conidiophore with the youngest nearest the apex. Sometimes only one spore is present, but frequently two or three attached spores are to be seen. Examination under high power of the microscope usually reveals scars on the conidiophore left by spores which have been detached. The conidiophore with its spores would, in terms of the inflorescence of a flowering plant, be described as a racemose spike. In the material I examined there were quite a number of conidiophores without any spores. It seemed to me that the fungus was nearing the end of its reproductive season.

Each spore is a specialized lateral branch-system consisting of a main axis (200–400 $\mu \times 3$–$4\,\mu$) with usually four (but more rarely three or two) lateral branches (50–200 $\mu \times 3\,\mu$). One or two of the lower members of these laterals may branch again to form one or two secondary laterals. At an early stage of development there is a cross-wall separating the spore-primordium from the conidiophore. Septa also occur in the branch system of the spore itself, but, in contrast to those of the conidiophore, they are difficult to see. The main axis of the spore tends to be bent at the point of departure of each lateral, and the same is true of the laterals which themselves branch. In nature the spore does not tend to occupy a single plane, although when mounted under a cover-glass it may become compressed into two dimensions.

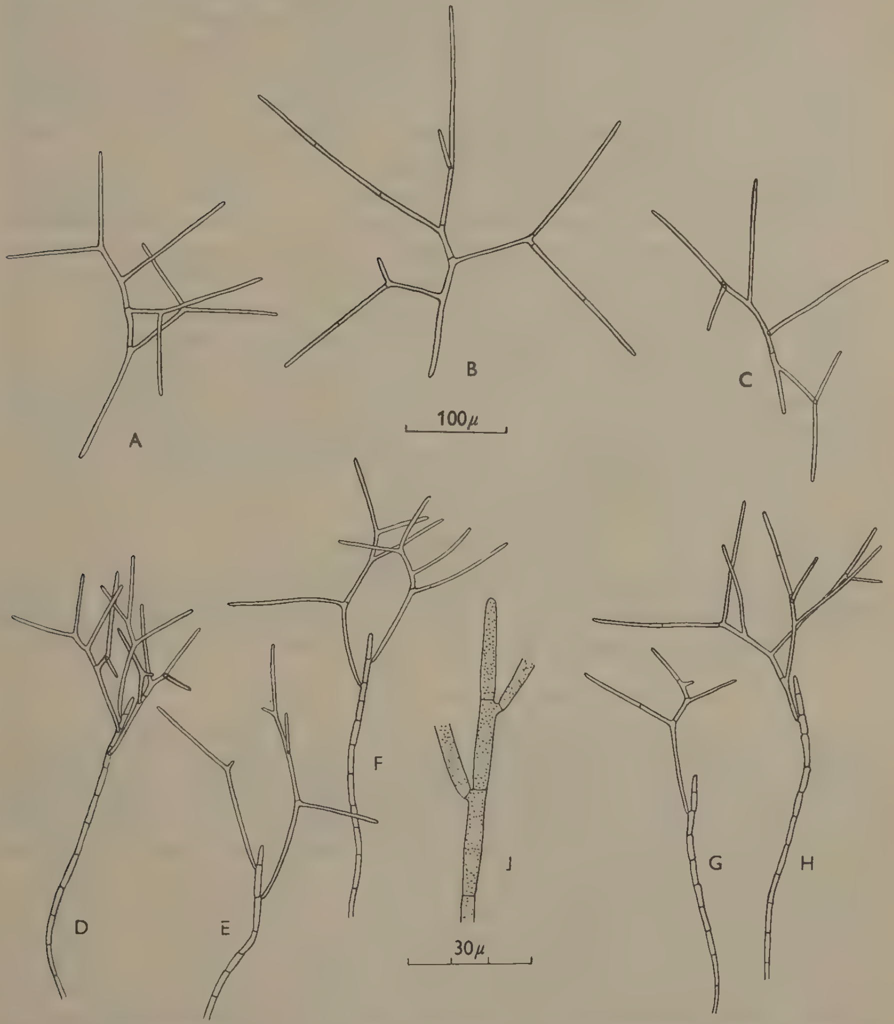

Fig. 1. *Polycladium equiseti.* A–C, liberated conidia; D–H, conidiophores bearing three (D), two (E, F) or a single (G, H) conidium. J, small part of F at higher magnification.

The taxonomy of this fungus raises certain problems. I thought at first that it might be assigned to the genus *Tricladium*, in which the spore consists typically of a main axis and two lateral branches arising at different levels (Ingold, 1942). This is the state of affairs in *T. splendens* (the type species), *T. angulatum* and *T. gracile*. I later extended the concept of the genus to include *T. anomalum* with a spore having a main axis bearing three laterals (Ingold, 1943). In the present species spores do occur without secondary laterals. In such spores the number of primary branches is usually four but may be three or even two. Spores with three laterals closely resemble those of *T. anomalum*. However, in all four species of *Tricladium* so far described, the spore is apical, and solitary at the end of the conidiophore (*T. splendens*, *T. gracile* and *T. anomalum*), or if there are several spores as in *T. angulatum*, the first to be formed is apical and represents the modified end of the conidiophore. Because the spore usually has secondary branches as well as primary, and because of the nature of the conidiophore with its continued apical growth, it seemed best to exclude the fungus from *Tricladium*.

Another possibility seemed to be to place it in the monotypic genus *Varicosporium*. In *V. elodeae* the spore is a branched system consisting of a main axis and one, two or three laterals, some of which may branch again (Ingold, 1942). In producing branched spores with a considerable range of form *Varicosporium* might seem a more suitable genus than *Tricladium*. However, in *Varicosporium elodeae* the relationship of the spores to the conidiophore is not closely similar to that of the species under discussion. In *V. elodeae* the first spore to be formed is terminal and subsequent ones arise lower down the conidiophore; there is no parallel with the conidiophore of the fungus on *Equisetum* with its apical cell. In view of these considerations it has been thought best to propose a new genus.

Polycladium gen.nov.

Fungi aquatici, submersi, mycelio septato, ramoso. Conidiophora simplex, ferens lateralia sessilia conidia, cum cellula apicali quae numquam fit conidium. Conidium (thallospora) hyalinum, ramosum, septatum, consistens ex principali filamentoso axe ferente plures ramulos quorum nonnulli iterum in alios ramulos diffunduntur. Typus:

Polycladium equiseti sp.nov. (Fig. 1)

Fungus aquaticus submersus, mycelio septato, ramoso. Conidiophora hyalina, simplex cum cellula apicali quae numquam fit conidium, 200–400 μ longa, 4–5 μ lata, cum conspicuis septis, ferens lateralia, sessilia conidia. Conidium (thallospora) ramosum, septatum (sed septis inconspicuis) consistens ex principali axe (200–400 μ longo, 3–4 μ lato) flexo qua oriuntur laterales ramuli, cum 4 (aliquando 2 aut 3) secundariis axibus (50–200 μ longis, 3 μ latis) et tertiariis axibus (aliquando absentibus) orientibus ex uno aut duobus secondariis axibus.

Hab. In submersis mortuis internodiis Equiseti fluviatilis in aqua jacentis in Blea Tarn Westmorland, Anglia. Typus: Herb. I.M.I. 73532.

My thanks are due to Prof. E. H. Warmington for his invaluable assistance with the Latin diagnosis and to Mr H. C. Gilson for allowing me to work in the laboratories of the Freshwater Biological Association on Windermere where this study was pursued.

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BRITISH RECORDS, 29–41

29. **Apodachlya minima** Coker & Leitner (*J. Elisha Mitchell sci. Soc.* **54**, 313, 1938) was found on submerged twigs of *Quercus robur* L. collected from: (*a*) ponds at Keele, Staffs., 12 March 1954; and (*b*) ponds at Dinas Powis, Glam., S. Wales, 22 Nov. 1955. Sporangia as described by Johnson (*Trans. Brit. mycol. Soc.* **38**, 415–418, 1955) were not found. It occurred in association with *A. pyrifera* and *Saprolegnia hypogyna*. There are only two previous records of *A. minima*, one from the United States and the other from the West Indies. In each of these cases it was found on hemp seed baits added to water and soil samples.

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30. **Apodachlya brachynema** (Hildebrand) Pringsheim (*Ber. dtsch. bot. Ges.* **1**, 289, 1883) was collected from: (*a*) ponds at Dinas Powis, Glam., S. Wales, on submerged twigs of *Quercus robur* L., 20 Feb. 1955 (Herb. E.P. No. Ap. 1); (*b*) lakes at Keele, Staffs., on grapes used as a bait, 4 Mar. 1955 (Herb. E.P. No. Ap. 2); (*c*) Doddington Mere, Ches., on submerged twigs of *Ulmus* sp. and *Fraxinus excelsior* L., 15 Mar. 1956 (Herb. E.P. No. Ap. 3); (*d*) Loch Lomond, Scotland, on twigs of *Betula verrucosa* Ehrh., 11 Aug. 1956 (Herb. E.P. No. Ap. 4). This species has been recorded once previously for Britain by F. K. Sparrow (these *Transactions*, **40**, 532, 1957) from Cambs. and Suffolk, on hemp seed bait and unnamed twigs.

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31. **Apodachlya punctata** Minden (*Kryptogamenflora Mark Brandenb.* **5**, 568, fig. 15, 1912) was found on submerged twigs of *Quercus robur* L. collected from: (*a*) ponds at Keele, Staffs., 12 Mar. 1954; and (*b*) ponds at Dinas Powis, Glam., S. Wales, 22 Nov. 1955. This species has not been previously recorded for Britain. The golden oospores were seen to possess thick walls which are distinctly and minutely punctate as described by von Minden and do not merely appear to be so because of oil-droplet arrangement as Coker & Leitner supposed (*J. Elisha Mitchell sci. Soc.* **54**, 313, 1938).

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32. **Apodachlya pyrifera** var. **macrosporangia** Tiesenhausen (*Arch. Hydrobiol.* (*Plankt.*), **7** (2), 295, fig. 19, 1912) was found on submerged twigs of *Quercus robur* L., collected from ponds at Dinas Powis, Glam., S. Wales, 26 Dec. 1956 (Herb. E.P. No. Ap. 5). This variety has not previously been recorded for Britain. It occurred in association with *A. pyrifera* Zopf, *Monoblepharis* spp. and *Gonopodya* spp.

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33. **Apodachlya seriata** Lund (*K. danske vidensk. Selsk.* **9**, 34, fig. 14, 1934) was collected from: (*a*) lakes at Keele, Staffs., on submerged twigs of *Acer pseudoplatanus* L., 23 Oct. 1954; and (*b*) ponds at Dinas Powis, Glam., S. Wales, on submerged twigs of *Quercus robur* L., 26 Dec. 1956. Sporangia were pyriform or subcylindrical, 35–90 × 15–25 (majority 50–60 × 20) μ, terminal on the main hyphae, sometimes on lateral branches, or in basipetal series. Zoospores 10–12 μ diam., escaping through a short apical exit tube, encysting in a hollow sphere at the mouth of the sporangium. This species occurred in association with *Monoblepharis* spp. and *Apodachlya brachynema*. Recorded only once previously, by Lund from Denmark on twigs of *Alnus*.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

34. **Araiospora pulchra** Thaxter (*Bot. Gaz.* **21**, 328, pl. 23, figs. 20–25, 1896) was found on submerged twigs of *Quercus robur* L., collected from ponds at Dinas Powis, Glam., S. Wales, 20 Feb. 1956 (Herb. E.P. No. Ar. 1). This species has been previously recorded only from the United States. These specimens resembled those described by Thaxter in every respect except for the absence of the spiny sporangia noted in the original description. Large numbers of smooth-walled, subcylindrical or broadly clavate sporangia, borne in whorls or umbels at the tips of segments, from which they are separated by a constriction, were present. This species occurred in association with *Monoblepharis* and *Gonapodya*.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

35. **Mindeniella spinospora** Kanouse (*Amer. J. Bot.* **14**, 301, pl. 34, 1927) was twice collected from Doddington Mere, Ches.: (*a*) in April 1955, on submerged twigs of *Ulmus* sp.; and (*b*) in May 1955, on tomato fruits used as a bait (Herb. E.P. No. Md. 1). Previous records show this species to have been collected only from the United States, on decaying, submerged, rosaceous fruits. Biflagellate zoospores as reported by Sparrow & Cutter (*Mycologia*, **33**, 291, 1941) were seen.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

36. **Rhipidium parthenosporum** Kanouse (*Amer. J. Bot.* **14**, 344, pl. 48, 1927) was collected from: (*a*) ponds at Dinas Powis, Glam., S. Wales, on twigs of *Quercus robur* L. and fruits of rose, 5 Apr. 1955; and (*b*) lakes at Keele, Staffs., on *Crataegus* fruits, 20 Oct. 1955. On rose fruits this species occurred in association with *R. americanum*. There is only one previous British record of *R. parthenosporum*, by F. K. Sparrow (*J. Linn. Soc.* (*Bot.*), **50**, 460, 1936) from Cambridge, on unnamed twigs. Mature parthenospores were spherical, smooth, thick-walled bodies completely filling the oogonia, as described by Sparrow.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

37. **Gonapodya prolifera** (Cornu) Fischer (*Rabenhorst, Kryptogamen-Fl.* **1** (4), 382, 1892). Sexual reproduction in *G. prolifera* as reported by Johns & Benjamin (*Mycologia*, **46**, 202–206, 1954) from the United States has not been previously recorded for Britain. Abundant material of this species showing gametangial formation was collected from: (*a*) ponds at Keele, Staffs., on rose hips used as bait, 22 Nov. 1954 (Herb. E.P. no. G. 1) together with *G. polymorpha* which was also showing gametangial stages; (*b*) Doddington Mere, Ches., on submerged twigs of *Quercus robur* L., 27 Oct. 1955 (Herb. E.P. No. G. 4); and (*c*) ponds at Dinas Powis, Glam., S. Wales, on submerged twigs of *Q. robur* L., 20 Feb. 1956 (Herb. E.P. No. G. 29) together with *G. polymorpha*, which was also showing gametangial stages. Sexual reproduction in this latter species was reported from this locality by F. K. Sparrow, these *Transactions*, **40**, 532, 1957.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

38. **Olpidiopsis vexans** Barrett (*Ann. Bot., Lond.*, **26**, 231, pls. 23–26, 1912) was collected from ponds at Dinas Powis, Glam., S. Wales, 9 Mar. 1957 (Herb. E.P. No. Ol. 1). It was found parasitizing hyphae of *Saprolegnia* sp., on submerged twigs of *Quercus robur* L. together with *Apodachlya pyrifera* Zopf, *Achlya radiosa* Maurizio and *Monoblepharis* spp. This species has not been previously recorded for Britain.

ELIZABETH PERROTT, *University College, Keele, Staffs.*

39. **Volucrispora aurantiaca** Haskins (*Canad. J. Microbiol.* **4**, 274, 1958). Spores agreeing both in size and shape with the highly distinctive conidia of this species were observed (Fig. 1) in scum from a small stream at Lullingstone, Kent, in Dec. 1955. In addition, the scum contained abundant spores belonging to twelve species of aquatic Hyphomycetes. Haskins found his fungus whilst isolating chytrids from soil, and suggests that it may either have come from soil or as a contaminant from the air. He considers that it is not an aquatic hyphomycete since 'when...the fungus is grown in water its spores are produced at or near the surface film and are released onto the surface film'.

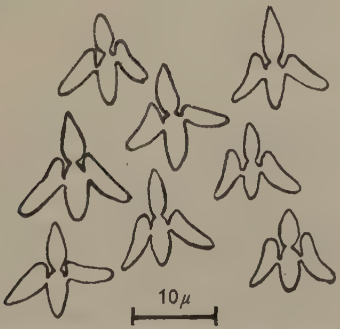


Fig. 1. *Volucrispora aurantiaca.* Spores from stream scum, Lullingstone, Kent.

C. T. INGOLD, *Birkbeck College, University of London*

40. **Alternaria sonchi** J. J. Davis apud J. A. Elliott (*Bot. Gaz.*, **62**, 416, 1916) was common on older leaves of a luxuriant *Sonchus oleraceus* plant at Cobham, Surrey, in Sept. 1958 (Herb. I.M.I. 74741 b). The leaves of this plant were also abundantly attacked by *Bremia lactucae* Regel. *Leaf spot* 2–10 mm. diam., more or less orbicular, somewhat vein-limited, greyish brown above with a narrow purple margin, rusty brown below without a purple margin. *Conidiophores* amphigenous, produced singly or in small groups or fascicles, pale to moderate (but not deep) olivaceous, smooth, septate, geniculate at the conidial scars, very short on the upper surface, longer on the lower surface, 20–75 × 7–10 μ. *Conidia* moderate clear olivaceous (rather deeper than the conidiophores), obclavate, broadly rounded at the base and gradually tapered to an obtuse apex, when young more or less smooth, when mature finely verrucose, with 2–8 transverse septa, some smaller conidia without any longitudinal septa but most with longitudinal or inclined septa in one or more cells, produced singly or rather rarely in chains, 36–95 × 9–24 μ (Fig. 1).

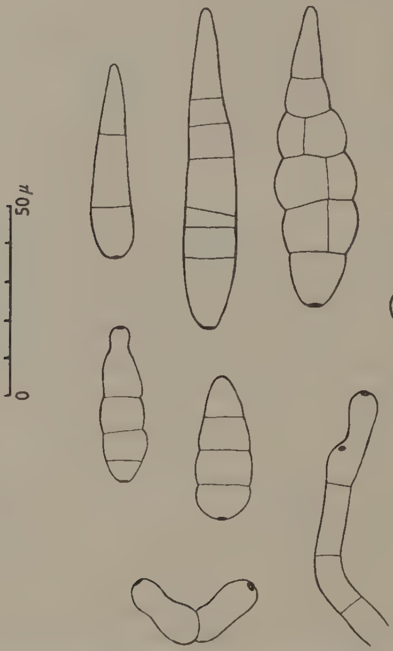


Fig. 1. *Alternaria sonchi*: conidiophores and conidia, from Herb. I.M.I. 74741 b. (× 500.)

This species is characterized by the short fat conidia, up to about 100 μ long and 24 μ wide, with a short apical beak, only rarely produced in chains.

A. sonchi was originally described from Wisconsin, U.S.A., on *S. asper* and on *Lactuca canadensis* on which leaf spots were produced by inoculation from the fungus on *Sonchus*, and is represented in Herb. I.M.I. by several collections on *Sonchus* and *Lactuca* from Uganda, Kenya, Republic of the Sudan and Cyprus, but does not seem to have been recorded before from Great Britain.

F. C. DEIGHTON, *Commonwealth Mycological Institute, Kew.*

41. **Pleospora betae** Björling (*Bot. Notiser*, 1944, p. 218), perithecial state of *Phoma betae* Frank (*Z. Ver. Rubenz. Indus. Dtsch. Reichs.* **42**, 904, 1892), as found on the dried stems of wild beet, *Beta vulgaris*, at Dunholme, Lincs. in Oct. 1957. The ascospores were grown and these formed pycnidia of *P. betae* in culture thus confirming Björling's work (1944, *loc. cit.*). The following is probably the first description of the species in English:

The *ascocarp* forms in the cortex and is typical of the Pseudosphaeriales. It is globose with a short apical papilla, 260–400 μ diam. and 200–260 μ high with a wall 35–40 μ thick. The *asci* are bitunicate, clavate with a rounded apex, and 85–120 × 14–16 μ, with eight obliquely monostichous to subdistichous ascospores. The *ascospores* are brown, ellipsoid to ovoid, and slightly constricted at the three transverse septa. When mature they normally develop a single longitudinal septum in each of the two central cells, and are 20·4 × 8·9 (18–25 × 7–10) μ. The external appearance of the

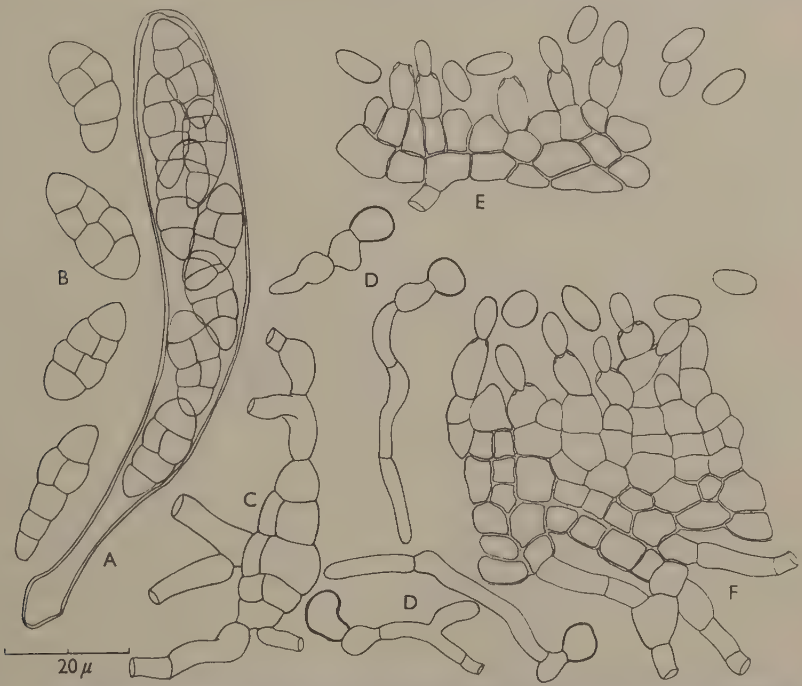


Fig. 1. *Pleospora betae.* A, mature ascus; B, ascospores; C, germinating ascospore; D, germinating pycnospores; E, segment of basal pycnidial wall from host; F, segment of lower pycnidial wall from 14-day culture.

pycnidium is similar to that of the ascocarp. They are 150–250 μ diam. and 200–230 μ high. The pycnidial wall is formed of two to three layers of irregular globose cells, 4–6 × 5–9 μ, with thick dark walls. The inner surface of the wall is covered with hyaline doliform phialides, 6–8 × 3·5 μ, and these form hyaline oval to subcylindrical *pycnospores*, 5–8 × 4–3 μ.

The pycnospores germinate in a manner not observed in other species of *Phoma*. The outer wall of the spore ruptures and the inner wall forms a sub-globose vesicle almost as big as the spore. From this vesicle, either the hyphal primordia develop straight away or another vesicle is formed first.

C. BOOTH, *Commonwealth Mycological Institute Kew*, and C. E. CORNFORD, *Dunholme Field Station, Lincs.*

Trans. Brit. mycol. Soc. **42** (1), 121–122 (1959).

OBITUARY

J. T. DUNCAN, F.R.C.S.Ir., D.T.M. & H.

1884–1958

Although Dr Duncan, who died unexpectedly on 3 June 1958, joined the Society in 1930 and was Vice-President in 1947, he was well known to few members. He rarely attended meetings and was even more rarely seen at a foray. His mycological interests lay in other directions. For a quarter of a century he did much to keep alive in this country an interest in fungi pathogenic for man and higher animals and many of the recent developments in this field in the British Isles can be traced to his researches, teaching and inspiration.

James Thompson Duncan was born at Dublin on 29 August 1884. He chose medicine as a profession and after a brilliant career as a student qualified from Dublin in 1911. Three years later he became lecturer in clinical medicine and pathology at King Edward VII College of Medicine, Singapore. He was Acting-Director of that College, 1916–18, and also held the part-time posts of Government Pathologist in the Straits Settlements and of consulting physician to the Tang Tock Seng Hospital, Singapore.

In 1919, as a result of ill health, Duncan returned to England where, after taking the Diploma in Tropical Medicine and Hygiene, he was appointed assistant lecturer in the department of tropical pathology at the London School of Tropical Medicine. In 1929 when this school was reorganized as the London School of Hygiene and Tropical Medicine he became lecturer in the new department of bacteriology, and Milner Fellow.

Duncan was then able to allow his mycological interests play. In addition to lecturing on tropical bacteriology he gave annual instruction to the Diploma of Bacteriology class on aspects of industrial mycology (a course subsequently taken over by Mr George Smith) and to this class and to that for the Diploma of Tropical Medicine and Hygiene he gave increasingly comprehensive accounts of mycotic infections and their pathogens. Over the years he built up a superb collection of teaching and reference material and the series of demonstrations to which each class was exposed were prepared with meticulous care by Duncan himself. The only criticism which could be made of these demonstrations, each comprising up to thirty or more carefully adjusted microscopic preparations supplemented by cultures, photographs, and summaries of the case histories, was that they made the diagnosis of mycotic infections seem almost too easy!

Duncan was a conscientious and fluent lecturer. He distributed cyclostyled notes (which were under constant revision) to ensure that a firm outline remained with each student and then enveloped the class in a torrent of fascinating detail. His academic career reached its climax two years before his retirement in 1949 when he was made the first University of London Reader in Medical Mycology, an appointment which is still unique both in this country and the Commonwealth.

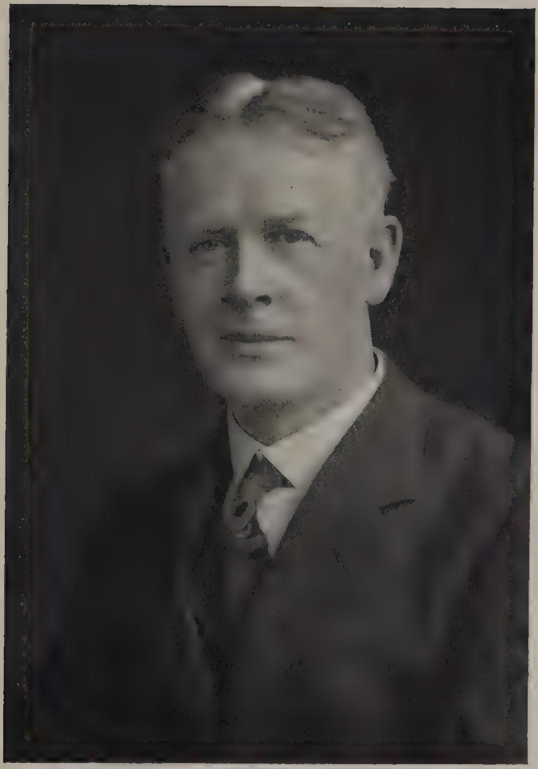
During the Second World War Duncan was seconded to the Public Health Laboratory Service and took charge of the Winchester laboratory. From there he made the first modern survey of mycoses in this country (see *Brit. med. J.* **2**, 715–18, 1948) and largely as a result of this the Medical Research Council established within the P.H.L.S. a Mycological Reference Laboratory at the London School of Hygiene and Tropical Medicine with Duncan as Director. The work of this laboratory (to which Dr Duncan recently had the gratification of seeing his daughter, Dr Jacqueline Walker, appointed Director) has done much to encourage an interest in mycotic infections of man in this country and to improve the accuracy of their diagnosis.

Duncan was a member of the Medical Research Council's Medical Mycology Committee from its inception in 1943 until his death; and he was its first chairman and subsequently served for a time as its secretary. He gave much assistance with the *Review of Medical and Veterinary Mycology* and for many years contributed valuable critical abstracts of publications on mycoses to the *Bulletin of Hygiene* and the *Tropical Disease Bulletin*.

Duncan had a deep interest in fungi, and all his work was based on long and painstaking laboratory studies of the pathogens and on a careful assessment of the literature. He made numerous original observations but this was coupled with a reluctance or inability (for he was in all his work a perfectionist) to publish his findings, which allowed others to forestall him in print. An occasional paper, a rare review, or a contribution to a symposium were the only published records of his mycological scholarship.

Though at times his impetuous Irish temperament made personal relationships difficult for him he was most generous to any interested student and had a wide circle of correspondents. The frequency with which authors in this country, in North and South America, in Africa, and in other parts of the world have made public acknowledgement of their indebtedness to Dr Duncan for cultures, pathological material, or good advice gives a truer measure of his stature than does his published work. It already seems clear that many of the seeds he sowed have fallen on good ground.

G. C. AINSWORTH



J. T. DUNCAN

PROCEEDINGS, 1958

MEETINGS

9 May 1958. Meeting held at Birkbeck College, University of London, at 11.0 a.m., The President, Dr F. B. Hora, in the Chair.

C. E. Yarwood. Some effects of rust fungi on their hosts.

Rust infection has been studied principally with the uredinial stage of *Uromyces phaseoli* on bean (*Phaseolus vulgaris*). The first effect noticed is stomatal closure within a few hours of inoculation. This may be responsible for the early but transient down-curling of heavily inoculated leaves. Later and with other rusts an up-curling of infected leaves may occur. The migration of host nuclei from the centre of epidermal cells toward the position of penetrated stomata is not peculiar to rusts, but occurs with other fungus infections. Light uredinial infection over an entire susceptible leaf or heavy infection in localized areas cause hypertrophies which are manifested by increased size of cells, but in a resistant host necrosis may result. Infection by basidiospores may cause hyperplasia. From the second to the fourth day after inoculation, infection may cause an increase in photosynthesis, but after this photosynthesis is decreased. Similarly, rust infection may increase the total dry weight of the above-ground parts of beans during the first few days of infection, but by 7 days after inoculation, the dry weight of infected plants is less than that of healthy plants. Rust infection increases respiration at any stage of infection. Transpiration is decreased up to about 7 days after inoculation, and then increased. There is a localized accumulation of starch around pustules. This extends beyond the rust mycelium and is greater for well isolated than for crowded pustules. This accumulation appears greatest in early morning, apparently because rust infection inhibits translocation of carbohydrates. It is apparently this local accumulation of carbohydrate which is responsible for the greater tolerance to heat shown by pustular tissue than by normal tissue, even though the rust mycelium is killed by the heat. Rusted tissue accumulates sulphur, phosphorus, carbon, and caesium in greater amounts than do healthy tissues. The higher pantothenic acid content of rusted than normal tissue is believed partly responsible for the greater nutritive value of rusted than normal tissue for snails and rats. Rust-infected tissues are more susceptible to some viruses, to powdery mildews, and to anthracnose than are normal tissues. When leaves which are rusted in localized areas are re-inoculated with the same strain of rust, with a different strain of the same species, with a different species of the same genus, or even with a species of a different genus, the second inoculation is specifically inhibited. The inhibitor is at least partially volatile and water soluble.

E. S. Griffiths. Cytological and cytochemical aspects of the differentiation of the microconidia of *Gloeotinia temulenta*.

Microconidia in *Gloeotinia temulenta* (as in other members of the Sclerotiniaceae) do not germinate and produce a vegetative mycelium: their function is purely sexual. The inability of the cells to germinate is attributed to the very low concentrations of ribose nucleic acid (RNA) in the cytoplasm which results from an elaborate pattern of cytological and cytochemical differentiation in the microconidiophores and associated cells.

The microconidiophore is sharply differentiated from purely vegetative cells by its low cytoplasmic RNA. Moreover its nucleus lacks a nucleolus, a structure known to be important for RNA synthesis, and a conspicuous feature of the nuclei of vegetative cells of *G. temulenta*. It is suggested that RNA synthesis is in some way inhibited in the microconidiophores and that these cells are dependent upon an external supply. In support of this view it has been shown that the cells subtending the microconidiophores show wide variations in cytoplasmic RNA—from very high to zero. Further, as long as RNA could be detected in the subtending cells, the microconidiophores invariably showed their normal low level. However, when zero levels were encountered in the subtending cells these were regularly associated with low or zero levels in the microconidiophores and the cessation of microconidial production.

The situation in *G. temulenta* has been compared with that in *Neurospora crassa* where the microconidia germinate and reproduce a vegetative mycelium. In this latter fungus very high values of RNA were found in both the microconidia and microconidiophores.

C. L. DUDDINGTON. Nematode-trapping Hyphomycetes and the control of eelworm.

The mechanisms by which predacious fungi capture eelworms are of two principal kinds: adhesive traps and mechanical snares. The commonest form of adhesive trap is the sticky network, consisting of a system of loops formed by anastomosing branches of the mycelium, to which eelworms adhere on contact. Another adhesive trap consists of a sticky knob borne on a short lateral stalk. The outstanding form of mechanical trap is the constricting ring, which consists of a three-celled ring borne on a short stalk; if an eelworm enters a ring the three cells swell inwards very quickly, gripping the eelworm firmly. In all forms of trap, capture of an eelworm is followed by the invasion of its body by trophic hyphae that grow out from the cells of the trap.

Predacious fungi are common in nearly all habitats where free-living eelworms are plentiful. They are found in rotting vegetable matter, in moss cushions, in dung and in the soil. They are easily grown on most ordinary laboratory media, and in pure culture without eelworms they do not usually form their characteristic eelworm traps.

Attempts to use predacious fungi for the control of plant parasitic eelworms were first made by Linford, working in Hawaii, on the pineapple root knot eelworm. He showed that there is a close connexion between activity of predacious fungi and the amount of organic matter in the soil, and his experiments indicated that the activity of naturally occurring predacious fungi in the soil could be stimulated to a useful extent by green manuring.

Experiments in this country on cereal root eelworm in oats have confirmed Linford's findings, and have shown that the initial invasion of oat seedlings by *Heterodera major* can be significantly reduced by the inoculation of the soil with predacious fungi coupled with the addition of organic matter, the combined treatment giving better results than either soil inoculation or green manuring by themselves. There is, however, considerable work to be done before large-scale use of the method can be feasible.

F. R. JONES. Some recent work on the Zoopagales.

The Zoopagales are a small group of delicate fungi which are obligatory feeders on small animals, mainly amoebae and nematodes, in the absence of which their culture has not yet proved possible. They occur in soil, fresh and brackish water, but are absent from soils where the acidity is above pH 5·5.

There are two main types of organization in the order; mycelial forms which feed by the intrusion of haustoria into amoebae or eelworms which happen to adhere to their hyphae; and thalloid forms, usually as short, thick, coiled hyphae, which are endoparasites of similar organisms. How the mycelial types capture the amoebae is not certain, but it is perhaps by adhesion to a sticky secretion of the hypha as does occur in the few more robust species attacking eelworms. With the endoparasitic forms, infection normally results from ingestion of the spores by the animal concerned; in some cases by spores adhering to the animal's pellicle which is later penetrated by the germ tube.

Reproduction is normally by conidia, which may be filiform or globose, and often have evacuated appendages at the distal end. These conidia are one-celled and produced singly, in groups, or in chains, almost sessile or elevated on long conidiophores. On these characters, and the form of the mycelium, is based the separation into the eight genera, of which only four or five are relatively common. *Cystopage* produces only chlamydospores.

The zygospores, resulting from the fusion of equal or unequal gametangia which arise from separate adjacent hyphae, are characterized by their pale yellow, regularly warted walls, and by the persistence of the 'zygosporangium' wall which may itself thicken, or remain as a thin sheath to the zygospore. Lateral conjugation, where both gametangia arise from a single branched lateral hypha, is occasionally a specific character, but in some species of *Stylopage*, lateral and normal conjugation habitually occur simultaneously in the same mycelium (*S. rhabdospora*). Azygospores and normal zygospores may also be produced in close association as a regular occurrence (*Zoopage cladosperma*).

H. G. Muller. The constricting ring mechanism of two predacious Hyphomycetes.

See these *Transactions*, **41**, 341–364, 1958.

REVIEWS

Club Root Disease of Crucifers caused by Plasmodiophora brassicae *Woron. A Monograph.* By John Colhoun. (Phytopathological Paper No. 3. Kew: Commonwealth Mycological Institute, 1958.) Pp. vi + 108, 4 plates. Price: 20*s*.

Dr Colhoun is to be congratulated on this detailed and accurate review of the literature, which covers all aspects of clubroot disease and its control, except the cytology of the pathogen, *Plasmodiophora brassicae*. Probably only those familiar with the literature of this disease will realize the appalling nature of this undertaking. It is hoped that many will appreciate the merits of the result.

There have been two previous monographs on clubroot, by Honig (1931: *Gartenbauwiss.* **5**, 116) and by Karling (1942: *The Plasmodiophorales*). Honig's review was combined with his own researches on clubroot, has an exhaustive bibliography but is now out of date. Karling monographed the whole group of organisms classified as Plasmodiophorales, giving an extensive treatment of their obscure cytology and detailed accounts of the two economically important diseases they cause, powdery scab of potatoes and clubroot of crucifers. The total volume of research on clubroot, in the last 16 years, is, however, scarcely great enough to require urgent summary. Hence, although the new review was evidently contemplated independently, it might be thought that the existence of Karling's monograph would have made it superfluous. That this is not so is due largely to the editorial blemishes of Karling's book. Mis-statements of fact, wrong references, and references omitted were faults that unfortunately marred an otherwise valuable work. This new review is carefully edited and thorough, besides being more complete in some respects, e.g. data on varietal reaction. A large number of quite useless references has been omitted from the bibliography. It is frustrating to find that a paper in some obscure journal, obtained with great difficulty, contains only the information that the cabbage crop in some remote province of Ruritania was devastated by clubroot in such and such a year. Yet the literature of clubroot includes many papers which tell little more. Dr Colhoun told me that the 400 references in his book were sifted from a list of 1500, a selection which is, in itself, a most useful contribution to the subject.

The main subject-matter is excellently arranged under the following headings: symptoms; life history; environmental factors in relation to infection; reaction of the host to infection; spore survival and dissemination; host range; varietal reaction of cultivated hosts to infection; physiologic specialization of the parasite; the nature of host resistance; control of the disease and methods for the evaluation of soil treatments. Each individual subject is usually dealt with historically or from field to laboratory. The list of headings and subheadings at the beginning of the book greatly facilitates reference. In particular, the organization of the section on control of the disease deserves special praise. There is, generally, a considerable amount of repetition, probably inevitable in a work of this kind, and one feels that the book could be shorter although this is perhaps less a question of literary conciseness than of approach. The author's intention has clearly been to summarize what research workers have stated rather than to assess its significance.

Very few omissions have been noticed. It is surprising, however, to find no reference to Kole's (1955: *Tijdschrift. PlZiekt.* **61**, 159) observations on fusion of zoospores from zoosporangia, which are important for the problem of the incompletely known life-cycle of *P. brassicae* and which could hardly be excluded as cytology any more than, say, descriptions of the flagellated stages. Again, although the early descriptions of infected buds are included, Walker's (1942: *Phytopathology*, **32**, 18) brief report of apical infection is not. This is unfortunate, because the migration of the parasite up the cambium from root gall to stem apex, recorded by Walker, is an excellent example of the ability of *P. brassicae* to move from cell to cell in the host plant, an ability which was disputed for many years. There are also instances of papers which are quoted but not all of the information in them is used, even when particularly relevant. A great deal of care has been expended on the compilation of the host range. Presumptive identification with authenticated generic and specific names is made of all names in the literature given without authorities. This is undoubtedly wise, and one might also wonder how carefully a plant pathologist who names a host plant with the authority for the name has really checked its taxonomy.

The style is clear and flows easily. There are some old-fashioned words such as 'malady' which seem out of place and the occasional expression such as 'an unnatural mode of entry for the parasite' (p. 14) which seems both quaint and paradoxical. There may soon be considerable excuse for the error of 'flagellae' (p. 13) for it is said and written so often that even those who started by knowing better may find it difficult to resist the pressure of common usage (Buchanan, 1958: *Bact. Rev.* **22**, 204). The plates are rather disappointing. Roots, in general, are difficult to photograph but I suspect that much of the quality of these pictures has been lost in reproduction.

These are minor criticisms and this book will be of great use to those already familiar with the organism and the disease. To those new to the subject or wishing to refer to it only occasionally the new monograph will be invaluable.

I. MACFARLANE

General Microbiology. By R. Y. STANIER, M. DOUDOROFF and E. A. ADELBERG. (London: Macmillan and Co., 1958.) Pp. xxii+682, 227 text-figures. Price: 50*s*.

To do full justice to the merits of this book in a review would require a writer of Caesar's economy, but one can begin by saying that the book, like Caesar's *Gaul*, is divided into three parts. Part I, entitled 'The Properties of Micro-organisms', is concerned with the various taxonomic groups—algae, protozoa, fungi, blue-green algae, bacteria and viruses—and with the autecology of bacteria, in the sense of physiology and genetics. Part I opens with three introductory chapters on microscopy and the discovery of micro-organisms, on the development of microbiological methods and on the role of micro-organisms in the living world. A treatment of the anatomy of the bacterial cell in ch. 7 prefaces chapters on all aspects of microbial physiology as exemplified by bacteria as the most intensively and extensively studied group of micro-organisms. The micro-biologists's microcosm includes photosynthetic activity (ch. 11), to the understanding of which a notable contribution has recently been made by Prof. Stanier and his research associates. Part I concludes with two brilliantly written chapters on the genetics, selection, adaptation and evolution of bacteria; the authors' remarks on the evolution and classification of bacteria on pp. 412–414 are worthy of careful consideration by all microbiologists. These two chapters, in which autecology merges into synecology, form a natural bridge with Part II of this book, entitled 'The Ecology of Micro-organisms', which is concerned both with microbial synecology and with the host-parasite relationship in its perfect and imperfect forms. The illustrations are chosen from amongst bacterial relationships, but this part of the book covers an extremely wide field of general interest to biologists. Part of ch. 21 is taken up by a fascinating account of the ruminant symbiosis; ch. 25 on acquired immunity in animals is a model of condensation for the general biologist. Ch. 26 on chemotherapy contains, *inter alia*, a notable illustration of the fact that the rational or scientific approach to a problem may not, in the short run, outyield the empirical approach. Ch. 27 on dynamics of disease in populations provides a useful definition for epidemiologists in need of a short answer (p. 526): 'Any sudden increase in the prevalence of a disease within a population constitutes an *epidemic*. There are epidemics of endemic diseases just as of nonendemic ones.'

The authors declare in their preface that 'This book is an attempt to present a modern synthesis of microbiological knowledge in a form intelligible to the beginner.' At least one middle-aged microbiologist has learnt so much from this book that he can acclaim the 'modern synthesis' as a remarkable feat of integration for the benefit of those with more than a beginner's background of microbiology. The 'beginner' will profit by first reading Part III, entitled 'The Biological Background', but time as well as effort is required to attain perspective, and most beginners will not quickly outgrow what they can learn from these pages.

In its ecological approach to problems both of microbiology and of micro-organisms as agents of disease, as well as in the choice of illustrations from animal rather than from plant pathology, this book challenges comparison with Sir Macfarlane Burnet's *Natural History of Infectious Disease*, published in 1953. Nevertheless, it is no disparagement to Sir Macfarlane Burnet's outstanding achievement to say that Professors Stanier, Doudoroff and Adelberg have no need to fear this inevitable comparison. Mycologists and plant pathologists have now less excuse than ever before for failing to know something about microbiology in general.

S. D. GARRETT

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